UNIVERSITY OF ALBERTA

The Synthesis of the Internal Disaccharide of Asialo GM_1 Carbon-13 Labeled at the Anomeric and Amide Centres

by

Lina Quattrocchio ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

Master of Science

Department of Chemistry

Edmonton, Alberta

Fall 1997

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **The Synthesis of the Internal Disaccharide of Asialo GM_1 Carbon-13 Labeled at the Anomeric and Amide Centres** submitted by **Lina Quattrocchio** in partial fulfillment of the requirements for the degree of **Master of Science**.

David R. Bundle, Supervisor

Derrick L.J. Clive, Examiner

Glen D. Armstrong, Examiner

Date: September 23rd, 1997.

To my husband Eduardo, my daughter Sandra and my sons Gabriel and Andres

To my parents Ada and Eugenio

Abstract

The recognition of mammalian cell surface oligosaccharides present as glycolipids and glycoproteins (glycoconjugates) is an important mechanism whereby viral and bacterial pathogens initiate infection of a host.

Pseudomonas aeruginosa, a gram negative bacterium, adheres to the cell-surface glycosphingolipid asialo GM_1 through the sugar binding sites of long filamentous protein structures called fimbriae or pili that protrude from the bacterial cell wall. Within the asialo tetrasaccharide, the key recognition element (epitope) is limited to the disaccharide βGalNAc(1→4)βGal. The same disaccharide also serves as the attachment site for the yeast, *Candida albicans*.

In order to probe the topology of the epitope's surface by NMR methods the βGalNAc(1→4)βGal disaccharide has been synthesized with selective ^{13}C labels.

A hexosaminyl-pentose disaccharide was assembled and subjected to a Kiliani-Fisher reaction, to give a mixture of two hexosaminyl-hexoses. The C-2 diastereomers, were resolved by chromatography of the corresponding per-acetate derivatives to give the target disaccharide, ^{13}C labeled at the reducing galactose residue. A disaccharide isotopically labeled at three sites was also synthesized by introducing double labeled acetate at the amino functionality of the hexosaminyl-pentose, followed by the Kiliani-Fisher reaction.

The mono- and tri-labeled disaccharides were converted to the corresponding allyl glycosides for subsequent attachment to protein or other carrier molecules that will facilitate NMR studies of the sugar-PAK protein complex.

Acknowledgments

I am very grateful to my supervisor, Professor David R. Bundle for his support, encouragement and understanding throughout my studies. I thank all the members in his research group for their help at different points specially Dr. Pavel Kitov for his advice in the laboratory work, Dr. Shahul Nilar for his dedication and patience in the elaboration of the posters and Joanna Sadowska and Carol Zheng for their friendship.

I would like to extend my thanks to the people of the spectral and analytical services departments, and especially to Dr. Albin Otter for his detail explanations about NMR, that gave me a better understanding of some of the techniques.

Financial support from the Protein Engineering Network Center of Excellence (PENCE) and from the University of Alberta is gratefully acknowledged.

Finally I would like to thank my husband Eduardo, my daughter Sandra and my sons Gabriel and Andrés for their support, encouragement and patience during this work.

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List of Abreviations

[α]	specific rotation
Ac	acetyl
Asn	aspargine
Asp	aspartic acid
Bn	benzyl
Bu	butyl
Bz	benzoyl
Cer	ceramide
DMF	*N*,*N*-dimethylformamide
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
ESMS	electrospray mass spectrometry
Et	ethyl
FABMS	fast atom bombardment mass spectrometry
Gal	galactose
GalNAc	*N*-acetyl galactosamine
Glc	glucose
GlcNAc	*N*-acetyl glucosamine
Gln	glutamine
Glu	glutamic acid
Ile	isoleucine
J	coupling constant
Lys	lysine
m	multiplet
Man	mannose
Me	methyl

MHz	megahertz
m.p.	melting point
Ms	mesyl (methanesulfonyl)
NeuNAc	5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosonic acid (*N*-acetyl-α-neuraminic acid)
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
Ph	phenyl
Phth	phthaloyl
ppm	part per million
Pro	proline
Py	pyridine
R_f	retention factor
r.t.	room temperature
Ser	serine
Tal	talose
Tf	triflate (trifluoromethane sulfonyl)
TFA	trifluoroacetic acid
Thr	threonine
TMSTf	trimethylsilyl triflate
Xyl	xylose

1.1 Introduction

Carbohydrates have traditionally been regarded as molecules that act as a source of energy, storage molecules or structural material. It was not until three decades ago that mammalian cells were shown to possess a sugar coat that was important for cell recognition processes[1-4]. Although carbohydrates hold the potential to form oligomers that are more complex than other biologically important molecules such as amino acids and nucleotides, their involvement in normal and abnormal cell-cell interactions attracted the interest of many research groups in the areas of chemistry, biology and medicine. Extensive studies, have shown cell surface carbohydrates to be essential for the binding of bacteria[5,6], viruses[7-10] and hormones[11] even playing a role in embryo development[12-15]. The fact that aberrant oligosaccharides have been found on the cell surface during the development and metastasis of tumor cells[16-19] reinforces the functional importance of complex glycoconjugates.

The polyhydroxylated nature of carbohydrates allows them to form a large variety of linear and/or branched structures with different ring sizes, different linkages and different glycosidic configurations. This diversity facilitates the high specificity of cell-receptor recognition. The wide range of biological activities involving naturally occurring carbohydrates[20] and some of their synthetic analogs, has encouraged scientists to study their potential use as drugs and conjugate vaccines, for example the anticoagulant heparin is currently in clinical trials for the prevention and treatment of thrombosis[21]. Other examples are the studies towards the discovery and development of drugs against the influenza virus[22-27] and vaccines for cholera[28,29].

1.2 Common types of cell surface oligosaccharides found in mammalian systems

The oligosaccharides present in the cell membrane can be covalently linked to either membrane associated proteins (glycoproteins) or lipids (glycolipids) and are both

now given the generic name, glycoconjugates. An important aspect is that glycolipids carry only one oligosaccharide per molecule while glycoproteins through more than one attachment site per molecule can carry several different saccharide chains linked to the same peptide sequence. Furthermore, the glycan chains of glycoproteins are branched and present at least two oligosaccharide chains and sometimes many more[30].

1.2.1 Glycoproteins

Most of the oligosaccharides linked to proteins are either *N*-linked to an amide nitrogen of aspargine or *O*-linked to the hydroxyl group of a serine or threonine[31]. There are others linkages such as those to the sulfhydryl group of cysteine[32] although they are less frequent. *N*-Linked glycans contain a common pentasaccharide core structure that is linked only to the specific sequence Asn-X-Ser/Thr where X is any amino acid other than proline[33]. This core pentasaccharide has the sequence α-D-Man-(1→6)-[α-D-Man-(1→3)]-β-D-Man-(1→4)-β-D-GlcNAc-(1→4)]-β-D-GlcNAc-Asn.

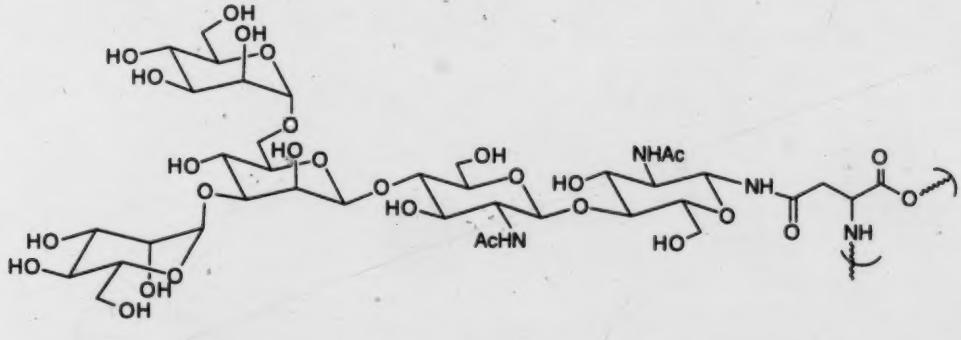


Fig 1. Pentasaccharide core common to *N*-Linked glycoproteins.

In contrast *O*-linked oligosaccharides do not have a common core structure and are not linked to specific amino acids sequences. Some reports point out that serine or threonine residues were found to be glycosylated more often with linkages such as GalNAc-α-Ser/Thr (Fig 2A, B)[34], GlcNAc-β-Ser (Fig 2C)[35], Xyl-β-Ser (Fig 2 D)[32],

Glc-β-Ser (Fig 2E)[36], and Fuc-α-Ser (Fig 2F)[36]. The GalNAc-α-Ser linkage is the most common.

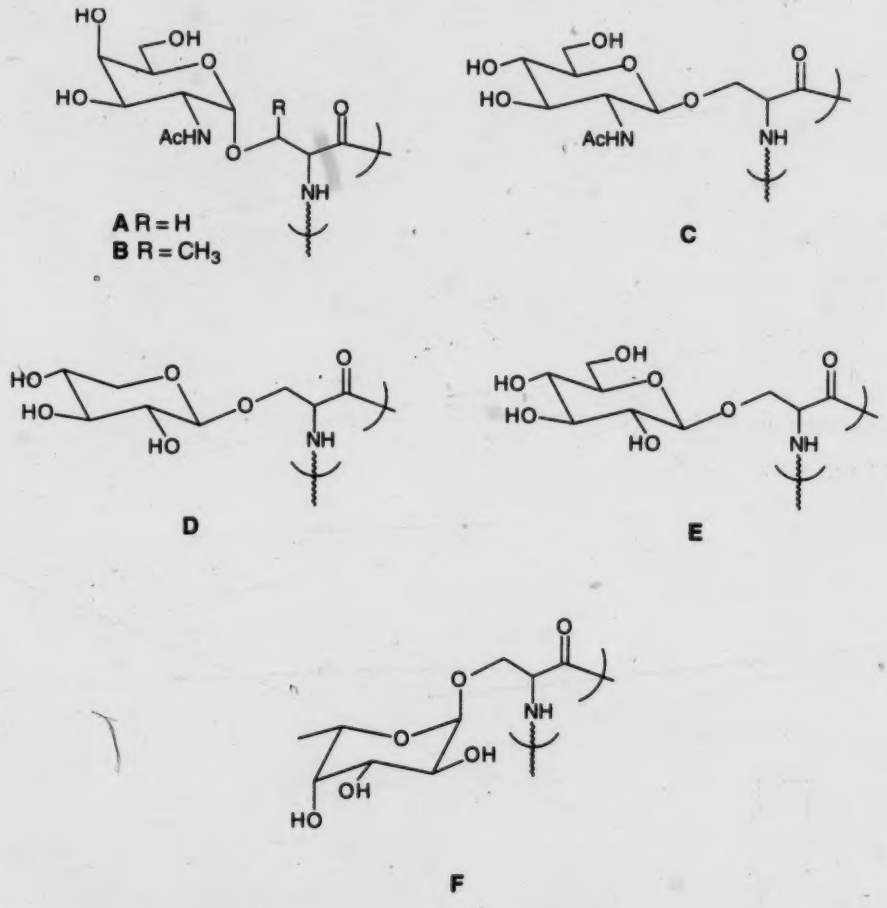


Fig 2. Examples of *O*-linked protein precursor.

1.2.2 Glycolipids

Glycolipids are sugar containing lipids that can be classify into two distinctive groups: those linked to ceramide[37] and those linked to phosphorylglycerol. In glycans linked to ceramide three building blocks can be distinguished: sphingosine (or a derivative), a molecule of a fatty acid and a carbohydrate residue. The sphingosine and

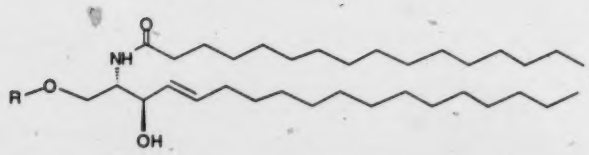
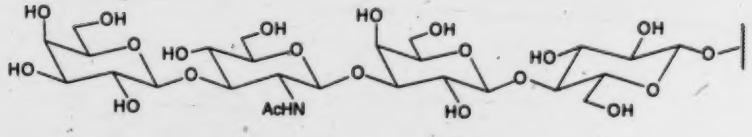
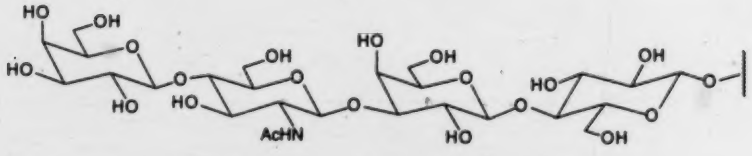


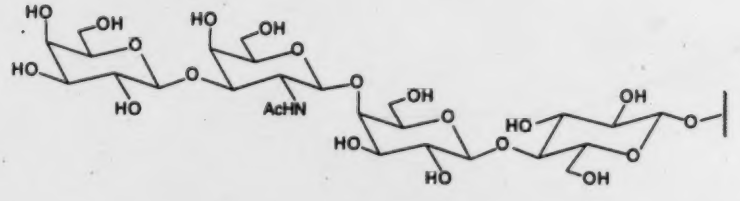
Fig 3. Ceramide structure. R = carbohydrate residue



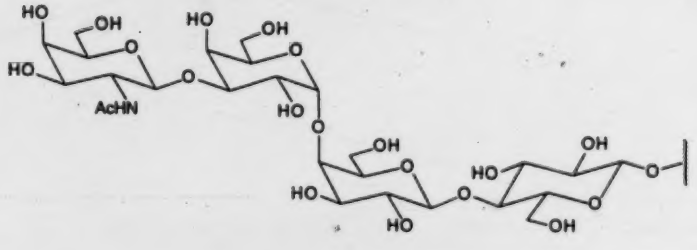
Lacto-series: Lactotetraosylceramide



Neolacto-series: Neolactotetraosylceramide



Ganglio-series: Gangliotetraosylceramide



Globo-series: Globotetraosylceramide

Fig 4. Characteristic glycolipid core structures.

the fatty acid form what is known as ceramide (Fig 3), the function of which is to anchor the sugar residue in the lipid bilayer of eukaryotic cell membranes.

Although it is difficult to classify glycolipids in a simple way, it is possible to recognize four characteristic core structures from which most of the glycolipids are derived (Fig 4).

The second group, the one in which sugars are attached to phosphorylglycerol (Fig 5), forms part of a linking arm for the attachment of proteins to membranes[38-41]. These structures have a phosphorylglycerol unit with two long chain alkyl or acyl groups that are embedded in the cell membrane. The oligosaccharide structure is bound to the phosphate via inositol, a six membered ring, and so far, it appears to have a core structure

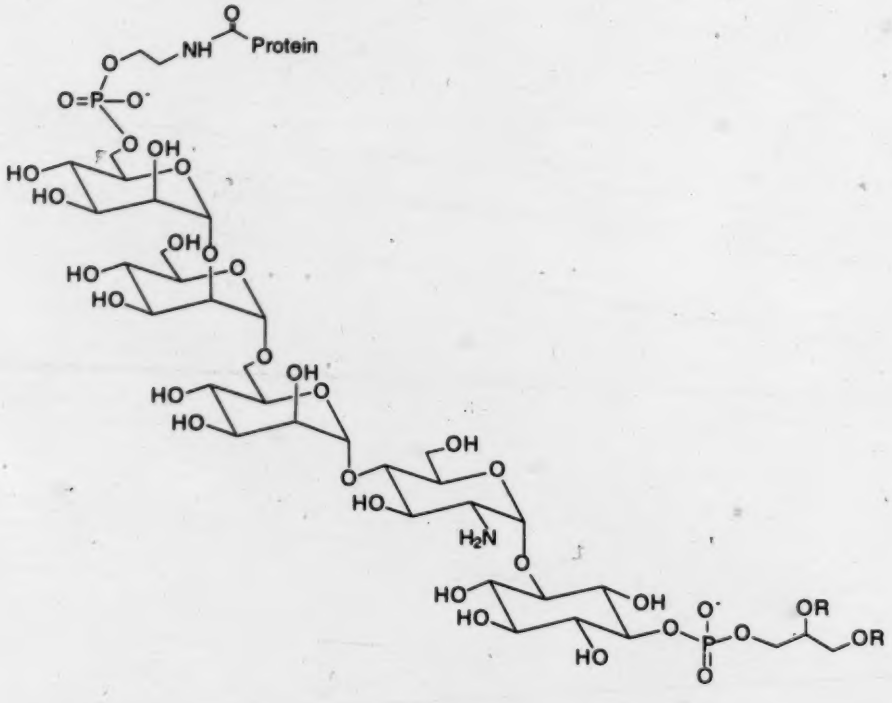


Fig 5. Common Core to glycerol phosphatidyl inositol anchors, R= long chain ester or alkyl groups.

consisting of glucosamine and three mannose residues. The protein is attached to the non-reducing end of the oligosaccharide through an ethanolamine-phosphate linkage.

1.3 Colonization and invasion of host surfaces

Bacterial pathogens cause disease by making use of mechanisms known as virulence factors[42,43] that allow them to accomplish their objective of colonizing the host-cell surface. In order to do this bacteria should be able to reach the host surface and adhere to it (Fig 6). A very general classification of the virulence factors based on their functions is presented below (Table 1).

Table 1. Virulence Factors and their functions.

Virulence Factor	Function
Pili, non-pilus adhesins	Adherence to host-cell
Enzymatic toxins, pore forming toxins	Neutralize host-cell
Capsules	Avoid phagocytosis
Variations in surface antigens	Evade antibody response
Invasins	Enter host-cell

Adherence is the first virulence factor and is an essential requirement in those areas such as the mouth, bladder, small intestine, etc., that present a mucosal surface in which the secretions act as washing mechanisms. The adherence takes place through adhesins also known as lectins present on the bacterial cell surface. These are basically of two different classes: a) pili or fimbriae or b) afimbrial adhesins.

Pili or fimbriae are rod-shaped protein structures packed in a helical array of 20 kDa molecular weight that protrude from the bacterial cell surface. The pili are long and relatively flexible structures. Their tip, is responsible for the adhesion to the host-cell

and, sometimes, has a distinctive protein structure. It is assembled with the aid of several proteins starting from the tip followed by the addition of pilin subunits that are pushed outside the bacterial surface. Due to the fragile nature of the pili, bacteria need to produce them constantly. This proves to be an effective way to avoid the host's immune response. *Neisseria gonorrhea*, for example, while maintaining its carbohydrate specificity, can change its pilus structure with such frequency that it is impossible for the host to mount an efficient antibody response[42].

Afimbrial adhesins are surface proteins that do not have a rod-like shape and that are believed to help the bacteria to bind tighter to the host-cell following the initial binding through pili[42]. As an example bacteria such as *Bordatella pertusis* have many adhesins and it is believed that distinct adhesins are used to adhere to different host-cell surfaces.

The oligosaccharides present on mammalian cell membranes as glycolipids and glycoproteins are the main target of bacterial adhesins[6]. The interaction between many copies of the protein receptor and the numerous carbohydrate epitopes on each endothelial cell is of low intrinsic affinity but develops high avidity by utilizing multipoint interactions.

The recurrence of increasing numbers of antibiotic resistant bacteria[44] has provided a strong impetus to the search for new antimicrobials and vaccines[45,46]. A better understanding of the mechanism of bacterial pathogenesis and the interactions taking place at the initial stage of the infection, are a focus of interest for the conception of new antinfective agents[45,46]. Interfering and blocking adhesion is one example of current thinking.

1.3.1 Blocking bacterial attachment

Bacteria become virulent in response to environmental stimuli and recent research is oriented towards the understanding of the mechanisms that trigger their virulence

factors[47], rather than reliance on antibiotics (Fig 6a). Once a bacteria turns virulent, one way of preventing infections could be to block the bacterial attachment (Fig. 6b). This could be done in two different ways: a) by delivering low molecular weight

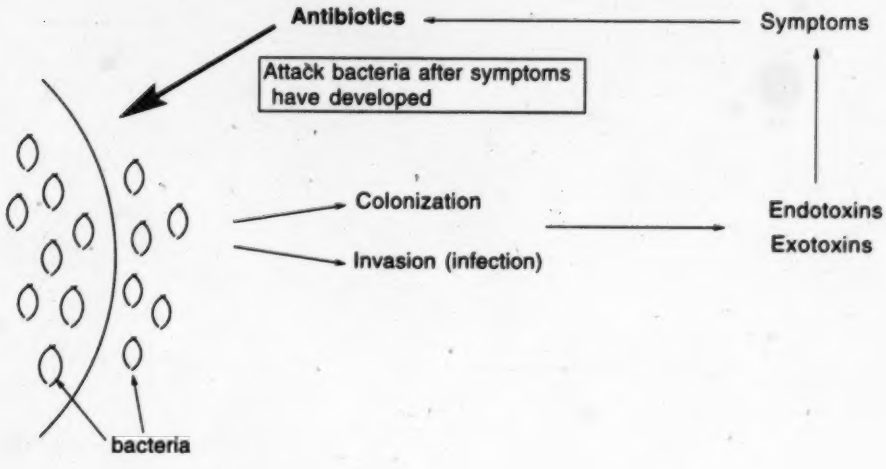


Fig 6a. Antibiotic therapy of bacterial infections.

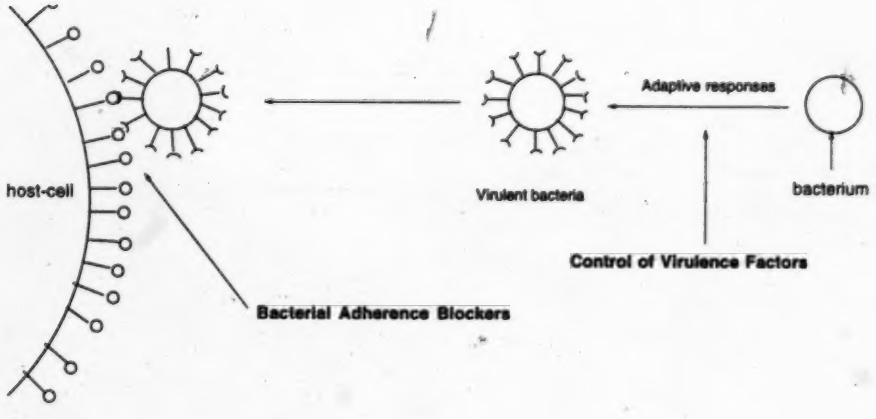


Fig 6b. Bacterial adhesion, an early stage for intervention.

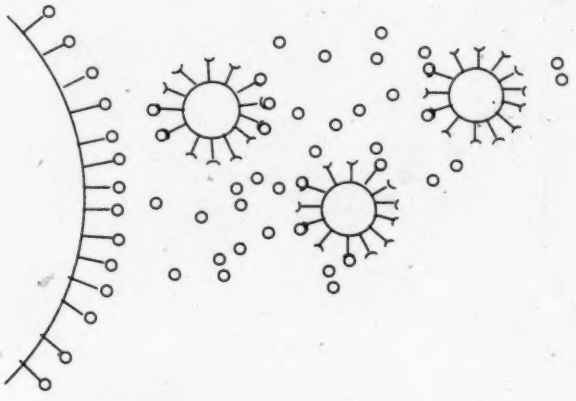


Fig 7. Blocking of the bacteria receptors.

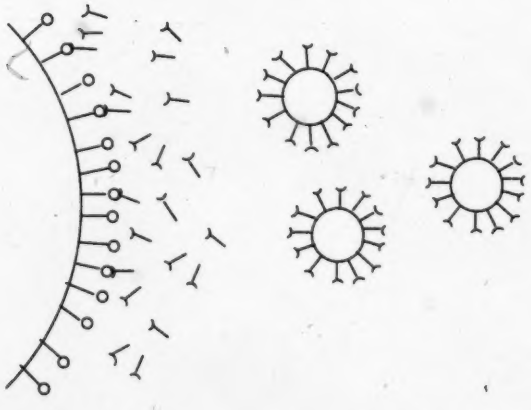


Fig 8. Blocking of the glycoconjugates on the cell surface.

oligosaccharides that will block the bacterial receptors (Fig 7) or b) by delivering soluble lectin-like molecules that will block the glycoconjugates on the cell surface (Fig 8)[42]. Already in 1979 simple sugar glycosides were shown to block bacterial adherence in the urinary tract[48], and years later, the successful use of antibodies against mannose to prevent certain *E. coli.* strains from infecting mice was reported[42]. For this approach to

be valid it is fundamental to know as much as possible about the donor and acceptor structures and the interactions taking place between them at the binding site.

Since protein crystalography has been successfully applied to only a small number of oligosaccharide-receptor complexes, it is unlikely that this will be a general method to reveal the molecular interactions in all oligosaccharide-protein complexes that have physiological significance. Consequently, chemical mapping studies have been developed to elucidate the molecular features of carbohydrate epitopes that are essential for recognition[49]. In many systems it is observed that two to three hydroxyl groups per oligosaccharide epitope are essential for binding, and their replacement by hydrogen or *O*-methyl groups shows a significant decrease or abolition of binding. For hydroxyl groups that exhibit such behavior it is inferred that their interaction with protein occur due to the formation of hydrogen bonds. In some cases water molecules mediate multiple hydrogen bonds between sugar residues and the protein surface[50,51].

1.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram negative bacterium and a serious pathogen for immunocompromised patients such as those with cystic fibrosis, burns and cancer. After colonizing the upper respiratory mucosal surface, the infection descends leading to the development of pulmonary disease[42]. Sequencing studies done on pili of *Pseudomonas* species isolated from patients with cystic fibrosis established that the adherence of this bacterium is mediated through a Type IV pilus that does not have separate adhesin molecules but a different amino acid sequence at the tip of the pilus. The pili of the PAK and PAO strains consist of pilin subunits with molecular weights of 15,000 Daltons arranged in a helix of five subunits per turn [52,53]. The carbohydrate binding domain peptide of the PAK pili is located at the C terminus and consists of a 17 amino acid sequence held by a disulfide bridge[53]. This peptide sequence recognizes various glycoconjugates including asialo GM_1[54,55] (Fig 9).

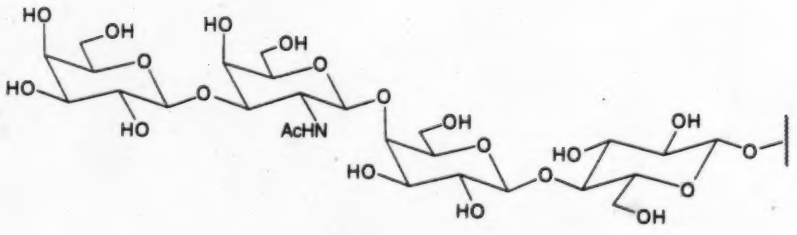


Fig 9. Structure of asialo GM_1.

Antibody recognition of this conserved loop showed that a seven residue peptide (134-140) was the minimum epitope required for maximum antibody binding. Of these seven amino acids Phe^{137} and Lys^{140} were criticals; Asp^{134}, Glu^{135}, Ile^{138} and Pro^{139} were important, and Gln^{136} was nonessential. The seven residue peptide mimicked the binding of the seventeen residue peptide suggesting therefore that the disulfide bridge was not critical for binding of the pilin to the antibody[56].

TLC based binding assays revealed that the bacterium binds to asialo GM_1 and asialo GM_2 that are known as isoreceptors[55]. It does not bind to galactosylceramide, lactosyl ceramide, globoside or Forssman glycosphingolipid which indicates that neither βGal nor βGalNAc alone are enough for binding[6]. Studies of bacterial adhesins to purified glycolipids lead to the conclusion that the bacterium recognizes the βGalNAc(1→4)βGal sequence both when present as a terminal or internal sequence of glycosphingolipids (Table 2)[6].

An interesting observation was the lack of binding to the gangliosides GM_1, GM_2, GD_{1a}, GD_{1b}, GT_{1d} and Cad, even though they have the βGalNAc(1→4)βGal sequence. It is proposed that the sialyl residue of these lipids interferes with the recognition process[6].

Additional studies found that other pathogens such as *Streptococcus pneumoniae*, *Staphilococcus aureus*, *Haemophilus influenzae*, *Klebsiella pneumoniae* and certain

Escherichia coli also recognize the same disaccharide sequence in glycolipids and gave positive inhibition with soluble disaccharides[6].

Table 2. Structures of some of the glycolipids tested for binding to *Pseudomonas aeruginosa.*

Asialo GM_1	βGal1-3βGalNAc1-4βGal1-4βGlc1-1Cer
Asialo GM_2	βGalNAc1-4βGal1-4βGlc1-1Cer
Galactosyl ceramide	βGal1-1Cer
Lactosyl ceramide	βGal1-4βGlc1-1Cer
Globoside	βGalNAc1-3αGal1-4βGal1-4βGlc1-1Cer
Forssman	αGalNAc1-3βGalNAc1-3αGal1-4βGal1-4βGlc1-1Cer
GM_1	βGal1-3βGalNAc1-4(αNeu5Ac2-3)βGal1-4βGlc1-1Cer
GM_2	βGalNAc1-4(αNeu5Ac2-3)βGal1-4βGlc1-1Cer
GD_{1a}	αNeuAc2-3βGal1-3βGalNAc1-4(αNeu5Ac2-3)βGal1-4βGlc1-1Cer
GD_{1b}	βGal1-3βGalNAc1-4(αNeu5Ac2-8αNeu5Ac2-3)βGal1-4βGlc1-1Cer
GT_{1b}	αNeuAc2-3βGal1-3βGalNAc1-4(αNeu5Ac2-8αNeu5Ac2-3)βGal1-4βGlc1-1Cer
Cad	βGalNAc1-4(αNeu5Ac2-3)βGal1-4βGlcNAc1-3βGal1-4βGlc1-1Cer

It was also found that mice infected with viruses that express a neuraminidase are susceptible to other pathogens. It was reasoned that viral neuraminidase may hydrolyze the sialic residue of glycolipids such as GM_1 (Fig 10) hereby exposing the βGalNAc(1→4)βGal sequence that can be recognized by other microorganisms including *Pseudomonas aeruginosa.* This provides one explanation for the sensitivity of patients to other microorganisms, after they have been exposed to a pathogen such as the influenza virus[6].

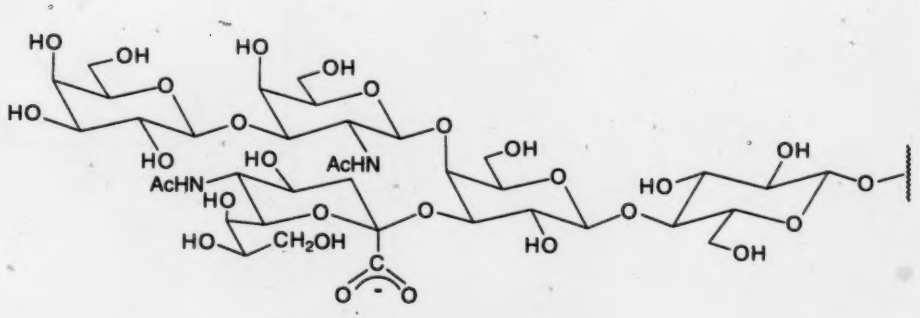


Fig 10. Structure of GM_1.

1.5 Objective of Research

Although the pilus of *Pseudomonas aeruginosa* has been extensively studied by protein methods, its interactions with glycolipids is only beginning to be appreciated. Epitope mapping studies with deoxy and mono-*O*-methyl analogs failed to show that any hydroxyl group was essential for binding[57]. This could imply that the interactions may be mostly of a hydrophobic nature. Examination of the preferred conformation of the disaccharide βGalNAc(1→4)βGal (Fig 11) reveals the bottom face of the molecule as the most hydrophobic. It is possible to assume that this face interacts with the protein.

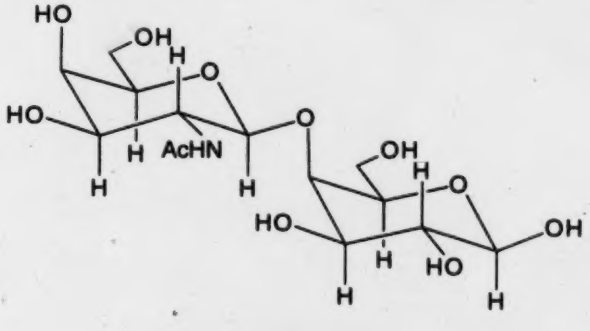


Fig 11. Target disaccharide.

At present, this is only a speculation, since it is not known to which surface of the oligosaccharide the pilus peptide binds. This thesis reports a synthetic approach to C-13 isotopically enriched disaccharide glycosides that could be used in NMR studies of

saccharide-pilus binding. NMR studies of the PAK pili protein bound to the labeled sugar disaccharide could be expected to provide some information about the pilus amino acid residues that come into contact with the disaccharide epitope.

1.6 General features of glycosylation reactions

The preparation of isotopically enriched oligosaccharides requires a labeling step and synthetic steps to assemble the oligosaccharides.

Monosaccharides such as those produced by the Kiliani-Fisher reaction have to be properly protected prior to synthetic strategies aimed at oligosaccharide synthesis. The protection steps are generally unavoidable and are a consequence of the polyhydroxylated nature of sugars. Although hydroxyl groups on pyranose rings have different nucleophilicities that permit selective glycosylation to be accomplished with sugars having several unprotected hydroxyls, this is not the general case. For the glycosyl acceptor, most commonly, it is necessary to follow a strategy in which all the hydroxyl groups are protected with the exception of the one to be glycosylated. Typically, this requires a 4 to 5 step synthesis. For the glycosyl donor, besides the protection steps it is essential to have a suitable activating group at the anomeric center, the synthesis of which requires an additional number of steps. The activating group functions as a leaving group generating an oxocarbenium ion that is susceptible to nucleophilic attack by the acceptor. This also implies a significant number of steps in order to have a proper monosaccharide donor[58-60].

The fact that nucleophilic attack on the oxocarbenium ion can lead to the formation of α or β glycosidic linkages (Fig 12), highlights the numerous variables such as temperature, solvent, polarity, catalyst, protecting groups, etc., that should be taken into account when a stereoselective product is desired. For example, lower reaction temperatures as well as the use of nitriles as solvents are known to favor the formation of β-glycosides.

Fig 12. Glycosylation reaction with a non-participating group at C-2.

For the formation of a 1,2-*trans* glycoside, as would be the case with the target disaccharide, it is common practice to take advantage of the protecting group at C-2.

Fig 13. Glycosylation reaction with a participating group at C-2.

Groups such as acetyl or benzoyl esters can act as participating or neighboring groups to form transitory acyloxonium ions that are opened by the acceptor from the *trans* side[60] (Fig 13).

In the case of 2-amino-2-deoxy hexoses 1,2-*trans* -glycosylation can be accomplished by introducing groups such as phthalimido that functions as a directing group due to its size and to its ability to stabilize the glycosyl carbocation by participation. Having assembled the disaccharide, the protecting groups are removed.

Non participating groups and relatively non-polar solvents favor 1,2-*cis*-glycosylation products[60-62]. Since products of this type are not the target for the synthesis of asialo GM_1 type structures, formation of 1,2-*cis* glycosides are not discussed.

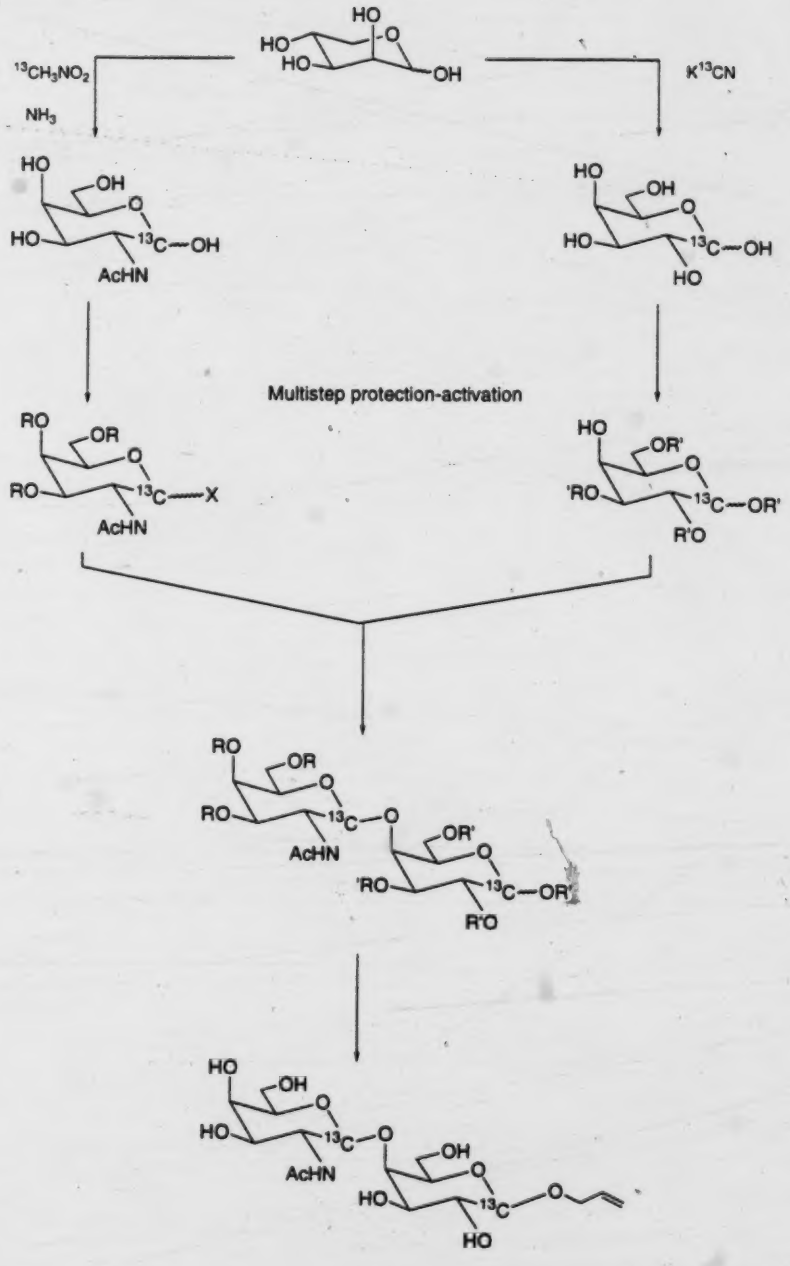


Fig 14. Less efficient use of labeled material.

1.7 Synthetic strategies for isotopic labeling

Basically, there are two different approaches that can be followed in order to obtain the selectively labeled GalNAc-Gal disaccharides shown in Figures 14 and 17.

The first one would label both monosaccharides at C-1 (Fig 14). This could be accomplished by synthesizing both ^{13}C-1 labeled monosaccharides (hexose and hexosamine) units starting from lyxose. The chemical reaction often used to introduce a ^{13}C label is the cyanohydrin reaction, first reported by Kiliani[63] and later modified by Fisher[64] and by Kuhn[65]. The reaction takes place by the nucleophilic attack of CN^- ions on the carbonyl carbon of an aldehyde or a ketone (Fig 15). For the purpose of this thesis

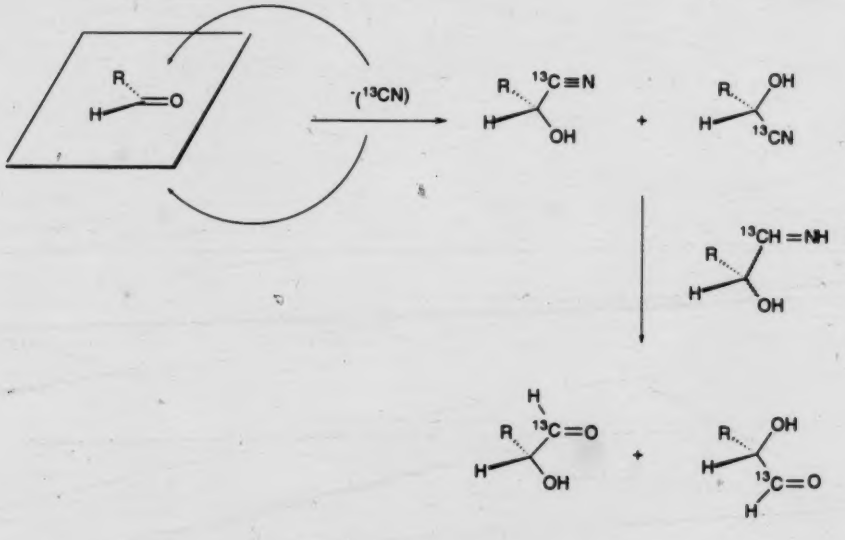


Fig 15. Kiliani-Fisher cyanohydrin reaction.

the interest was centered on the reactions of aldehydes since the target sugar is one carbon atom longer than the starting material. The cyanohydrin so obtained is subsequently reduced to the imine which hydrolyses spontaneously to the corresponding aldehyde (hexose)[66-68]. The two C-2 diastereomers (galactose and talose) are produced

in different yields and can be separated and isolated by chromatography on a cationic resin[69-70].

To synthesize a ^{13}C-1 labeled hexosamine residue starting from lyxose, another known method can be followed such as the addition of nitromethane to an aldehyde under basic conditions[71] (Fig 15). Once a nitrosugar is formed, the mixture of diastereomers is acetylated, followed by treatment with ammonia in methanol. This reaction introduces an amino group at C-2 of the nitrosugar and the generated C-2 diastereomers are then isolated by recrystallization. The desired C-2 nitrosugar is then reduced to give the 2-acetamido-2-deoxy-(^{13}C-1) monosaccharide[72].

The two ^{13}C-1 labeled monosaccharide residues have to be protected and activated for the formation of a β1,4-linked disaccharide, which requires approximately 10 steps, followed by the removal of the protecting groups, after the formation of the glycosidic linkage (Fig 16).

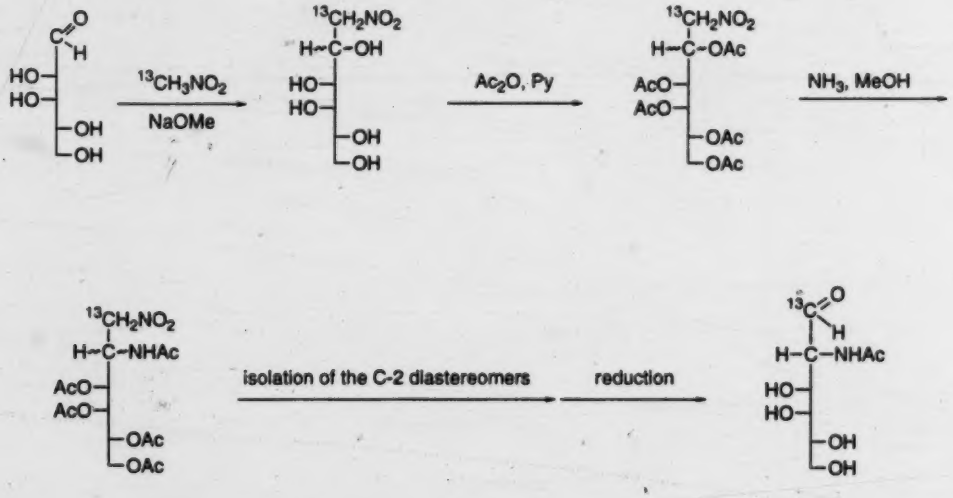


Fig 16. Synthesis of a hexosamine by nitromethane addition.

In the second strategy (Fig 17), the starting materials are glucosamine (hexosamine) and lyxose (pentose). Both sugars have to be protected and activated as a suitable donor and acceptor. When protecting the acceptor for the glycosylation, it is necessary to keep in mind that the disaccharide to be obtained has to have a β 1,3-linkage, since after a chain elongation by the cyanohydrin reaction, it will become a β 1,4-linkage. After coupling of the two sugars, the glucosamine-lyxose disaccharide can be transformed into a galactosamine-lyxose disaccharide through a nucleophilic displacement at C-4'. After removal of the protecting groups, the disaccharide is ready for the introduction of the label through the cyanohydrin reaction.

The first approach places the most expensive step, introduction of the isotopic label, at the beginning of a multi-step synthesis. Consequently a series of intermediate yield reactions can magnify the effective cost of the synthesis by a factor of 10 to 100. The alternative route introduces the ^{13}C label at the latest possible stage, but has the disadvantage of labeling only one of the sugar units (galactose). This deficiency can be addressed in part by the introduction of a ^{13}C labeled acetyl group at the amino functionality of the galactosamine residue. It has the additional potential that the final disaccharide in addition to a C-1 labeled hexose residue may be labeled at a methyl (δ ~ 23 ppm) and a carbonyl carbon (δ ~ 170 ppm).

This second more conservative approach, with a better use of the expensive labeled material, was the one followed in this project.

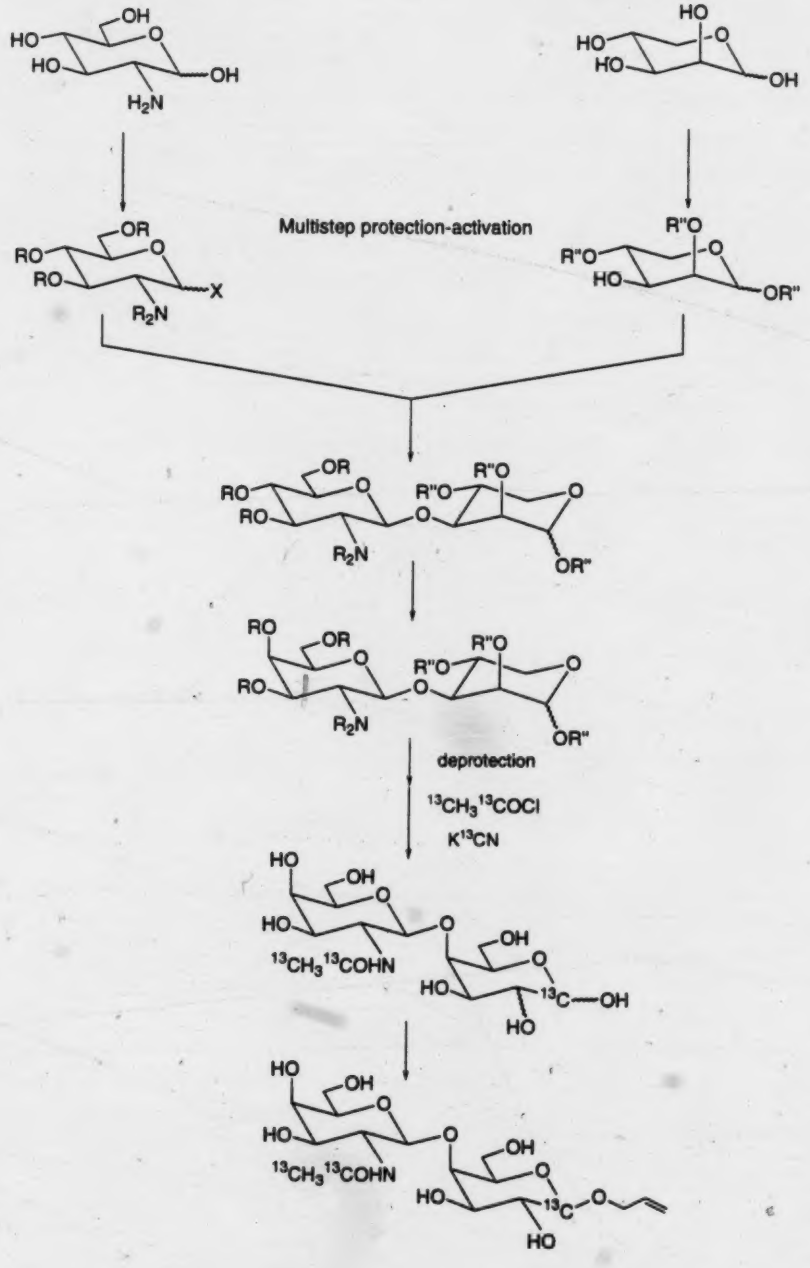


Fig 17. Conservative use of labeled material.

2 Results

The 2-amino-2-deoxy glucosamine donor was synthesized starting from glucosamine hydrochloride (**1**) (Fig 18) which was treated with sodium methoxide to give a supersaturated solution of glucosamine **2** and a precipitate of sodium chloride. After removal of the sodium chloride, the filtrate (**2**) was reacted with phthalic anhydride and triethylamine forming a crystalline solid of the triethylamonium salt of 2(2′-carboxybenzamido)-2-deoxy-D-glucopyranose. Acetylation with acetic anhydride in pyridine gave a 1:2 α/β mixture of the corresponding phthalimido per-acetates (**3** and **4**). The β acetate **4** was converted to the glycosyl bromide (Fig 19) by treatment with a saturated solution of hydrogen bromide in glacial acetic acid giving compound **5** as a 1:1 α/β mixture[62].

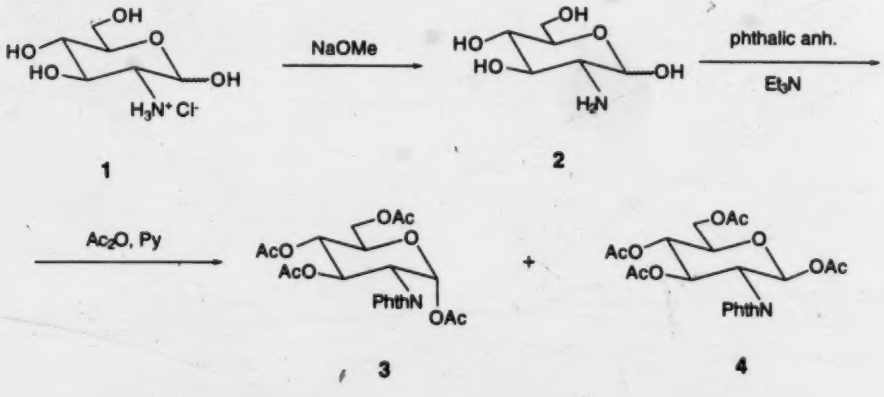


Fig 18. Preparation of the peracetylated glucosamine.

Glycosidation of compound **5** with *t*-butyl alcohol was carried out in dichloromethane using a mixture of silver salicylate and silver triflate as catalysts to give the *t*-butyl glycoside **6** in 70% yield[73]. The *t*-butyl-glucopyranoside (**7**) was obtained after removal of the acetates with sodium methoxide in methanol followed by neutralization with Rexyn 101 (H^+). The trihydroxy derivative **7**, was selectively

dibenzoylated at the 3 and 6 positions (**8**) by treatment with 2.5 moles of benzoyl chloride in pyridine[74].

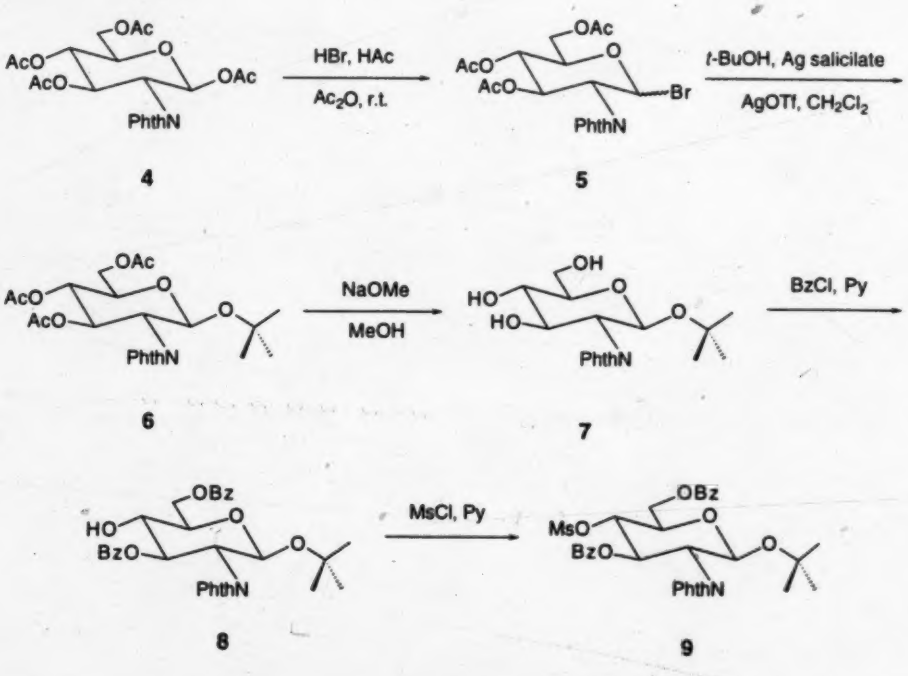
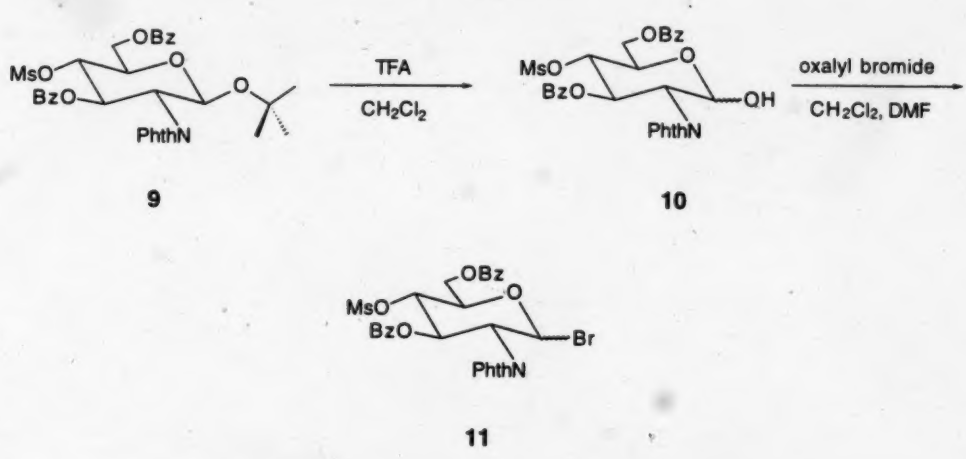


Fig 19. Preparation of the methylsulphonyl ester **9**.

Because the target disaccharide contains a galactosamine residue, the C-4 hydroxyl group of the glucosamine compound **8** had to be protected with a suitable leaving group so that it could be transformed into a protected galactosamine sugar by an S_N2 displacement. The chosen leaving group was the methanesulphonate ester which was introduced by treatment of **8** with methanesulphonyl chloride in pyridine, to give the methylsulphonate ester **9** as a foam. This glycoside **9** was converted to a glycosyl bromide by first removing the *t*-butyl group (Fig 20). This was done by treating **9** with TFA to give the reducing sugar **10**, almost exclusively as the β anomer.

Two procedures were tried in order to obtain the glycosyl bromide **11**; in the first one the reducing sugar **10** was reacted with oxalyl bromide[75]. The second one involved

treatment of compound **9** directly with HBr/HOAc[74]. The first procedure gave better yields, although it required an extra hydrolytic step.



9 **10** **11**

Fig 20. Preparation of the bromo glycoside **11**.

For the acceptor it was necessary to plan a synthesis leading to a lyxose derivative with a protecting group at the 3-position that could be selectively removed in the presence of persistent blocking groups. The route chosen targeted the synthesis of a 3-*O*-allylated, perbenzylated molecule, from which the allyl moiety could be removed by isomerization to the propenyl ether followed by hydrolysis (**17**).

Literature chemistry for selectively protected lyxopyranosides is limited. Therefore the choice of a protection strategy was based on stereochemical similarities with rhamnose and mannose. Lyxose is a pentose sugar which exists in solution as a 70:28 mixture of the α:β pyranose forms, with only 2% of the respective furanose forms[76,77]. In mannose and lyxose, the methyl α-glycoside is formed preferentially in the Fisher glycosidation reaction and these conditions provide a convenient method to obtain benzyl lyxopyranoside (**13**) (Fig 21). The use of cationic resin as acidic catalyst afforded poor yields (less than 45%), due to the relatively mild acidity of the resin and

the possible presence of some water in the resin[78]. The best result was obtained when acetyl chloride was used to generate HCl *in situ* affording compound **13** in 61% yield.

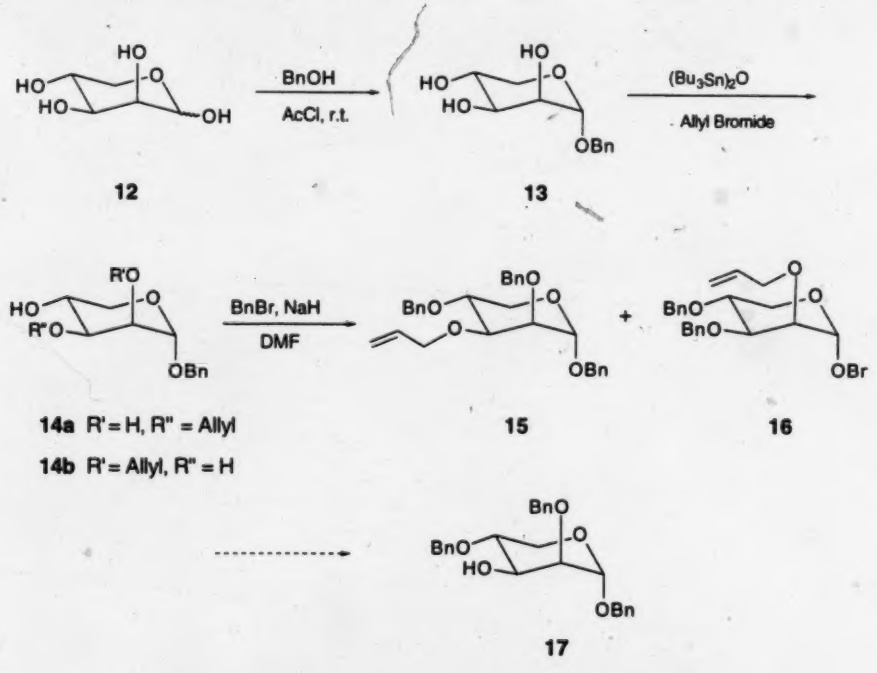


Fig 21. Preparation of the 3-*O*-allylated lyxose derivative.

Introduction of the allyl group was accomplished by regioselective methods. Regioselective alkylation of sugars via trialkylstannylation has been extensively reported though not for lyxose. Studies done on monosaccharides such as mannose and rhamnose that also have hydroxyl group pairs in *cis* axial-equatorial and *trans* di-equatorial arrangement, concluded that it was possible to allylate at the 3 position in a regioselective manner with yields ranging between 42 and 50%[79-81]. Similar procedures were then applied to benzyl-α-D-lyxopyranoside **13** in order to obtain **14**. The ^{1}H NMR at 360 MHz spectrum of the isolated reaction product **14** was difficult to assign with certainty. Taking into account a subsequent benzylation step of the remaining hydroxyl groups to give **15** and **16**, a small scale benzylation reaction was carried out

as a way to identify the compounds of the previous reaction. ESMS of the tribenzylated derivative showed peaks at m/z 499 $[M+K]^+$ and 483 $[M+Na]^+$ consistent with a molecular weight of 460 for $C_{29}H_{32}O_5$. The ^1H NMR spectrum clearly revealed the presence of two products through the anomeric hydrogens at δ 4.82 and 4.81 with coupling constants of 2.8 and 2.3 respectively. Based on published data for mannose, compounds **15** and **16** are probably the two monoallylated derivatives. Integration of ^1H NMR spectra indicates the formation of the 3-*O*-allyl and 2-*O*-allyl compounds in a 4:7 ratio. The fact that these products as well as the ones from the previous step had similar R_f values in TLC, indicated the need to look for another synthetic path.

Protection of **13** via acetonation of the *cis* C-2 and C-3 hydroxyl groups (Fig 22) permits the C-4 hydroxyl group to be benzylated followed by removal of the

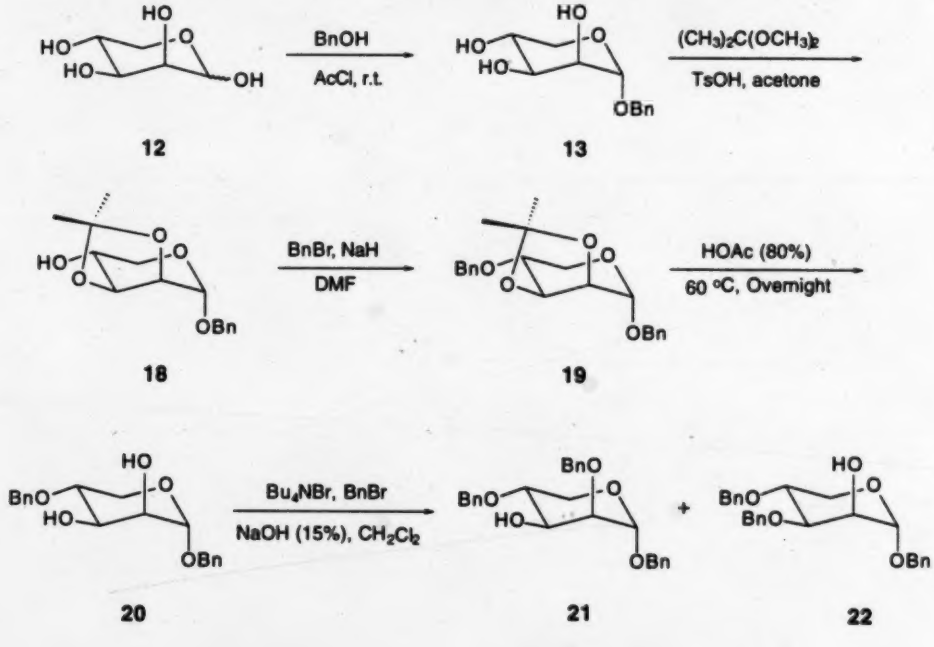


Fig 22. Preparation of the tri-benzylated donor **21**.

isopropylidene acetal to generate a 2,3 diol. The C-2 hydroxyl group may then be protected by a final phase transfer benzylation.

The 2,3-isopropylidene derivative **18** was obtained by treatment of the benzyl lyxoside **13** with dimethoxypropane in acetone. Compound **18** was benzylated with benzyl bromide in the presence of sodium hydride to give the benzylated isopropylidene lyxopyranoside **19**. After removal of the isopropylidene acetal with TFA, the diol **20** was benzylated under phase transfer conditions[82] to give a mixture of three compounds. The product present in smallest amount was identified as perbenzylated lyxose. In order to be able to identify the other two products, a small scale acetylation of the mixture was done (Fig 23). The major component was shown to be the 3-O-acetyl derivative **23** since the H-3 resonance at δ 5.26 ppm had small and large coupling constants ($J_{2,3}$ 3.4 Hz, $J_{3,4}$ 8.8 Hz), while the other product, the 2-O-acetate **24**, gave H-2 at δ 5.36 with two small couplings constants ($J_{1,2}$ 2.3 Hz and $J_{2,3}$ 3.1 Hz).

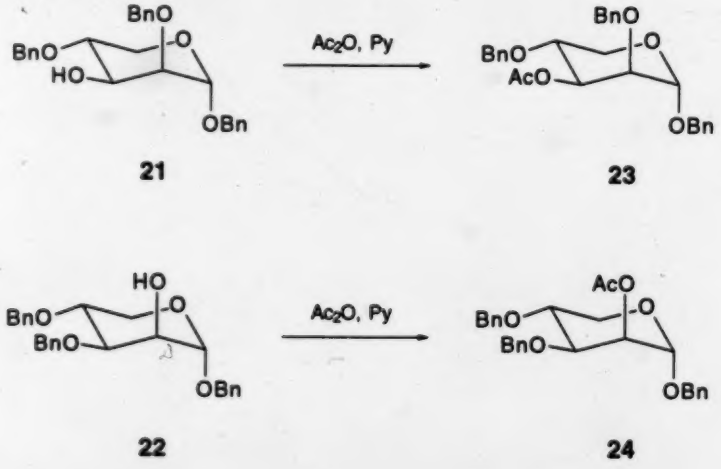


Fig 23. Preparation of the acetylated derivatives **23** and **24**.

Having synthesized both the glucosyl bromide **11** and the tribenzylated acceptor **21**, a glycosylation reaction was attempted (Fig 24) using silver triflate as an activator in

nitromethane, but no product was obtained. Another attempt used mercury cyanide and mercury bromide in acetonitrile. After worked-up, the products recovered were the hydrolyzed donor (**10**) and the acceptor (**21**). In order to verify the possible unreactive

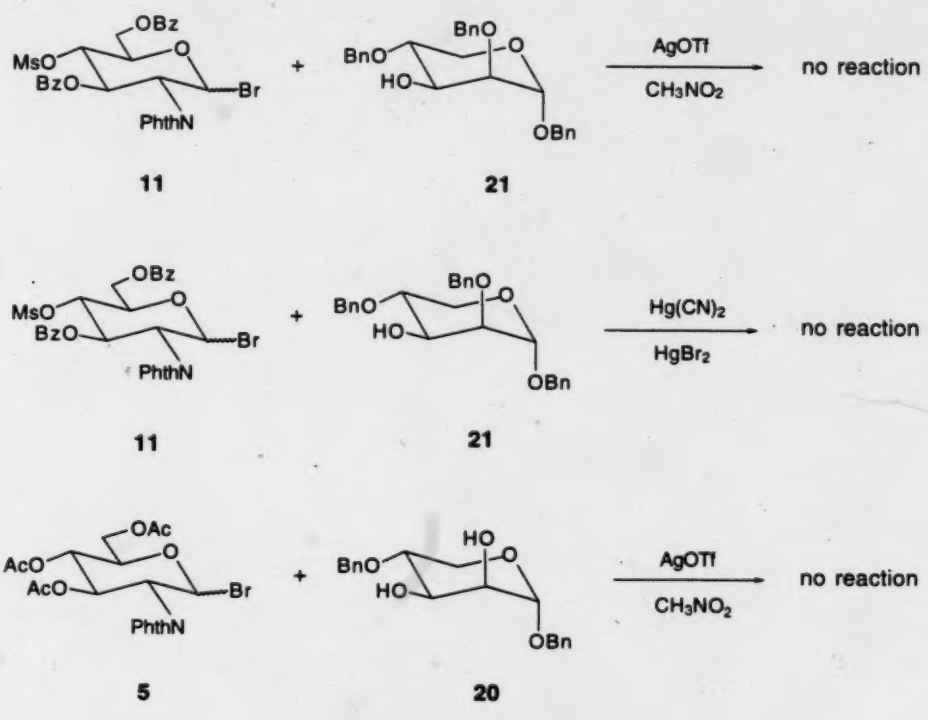


Fig 24. Gycosylation attempts.

character of the acceptor towards the donor, the coupling was tried between the glucosyl bromide **5** and the diol derivative **20** using silver triflate. Again the reaction gave only hydrolyzed donor. It was concluded at this point that the reaction should be tried using other glycosyl activators such as trichloroacetimidates or thioglycosides. The trichloroacetimidate was chosen as the first option and it was synthesized from compound **10** following the procedure described by Schmidt[83] (Fig 25). The reaction to form the trochloroacetimidate was fast and after concentration the residue was chromatographed to give the glucosamine imidate **25** in 87% yield.

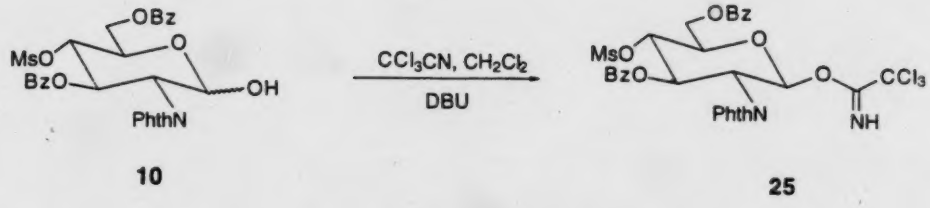


Fig 25. Preparation of the glucosamine imidate **25**.

A glycosylation reaction was then carried out between the glucosamine imidate **25** and the diol **20** with the expectation of some selectivity towards the desired 3 position (Fig 26). One dimensional ^{1}H NMR spectroscopy of the two products obtained was not

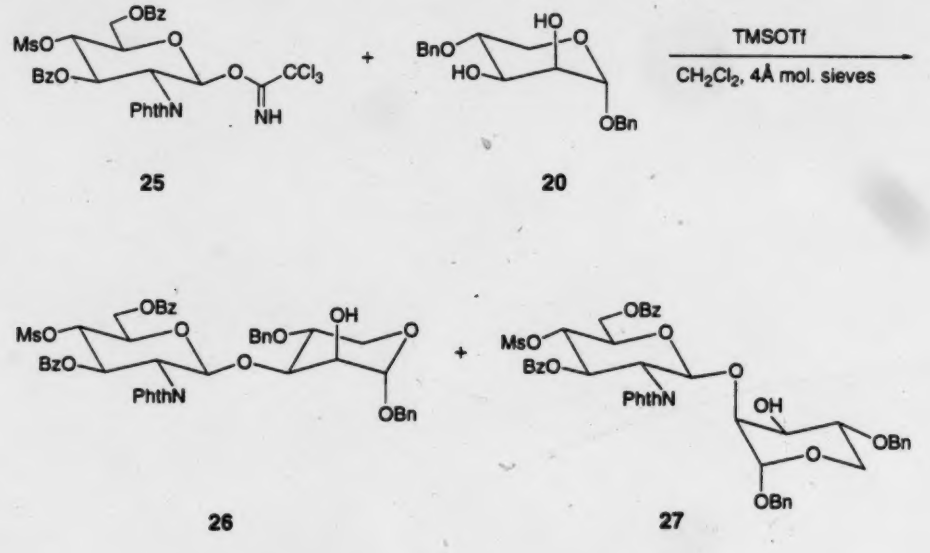


Fig 26. Synthesis of a disaccharide using diol **20**.

sufficient to unambiguously identify the two products obtained. The assignment could be made from a one dimensional version of a two dimensional NOE experiment (1D-TROESY) that used a selective pulse applied at H1′. This achieves a through space correlation from protons that are close (< 3.0 Å). The H-2 resonance of the 1,2-linked

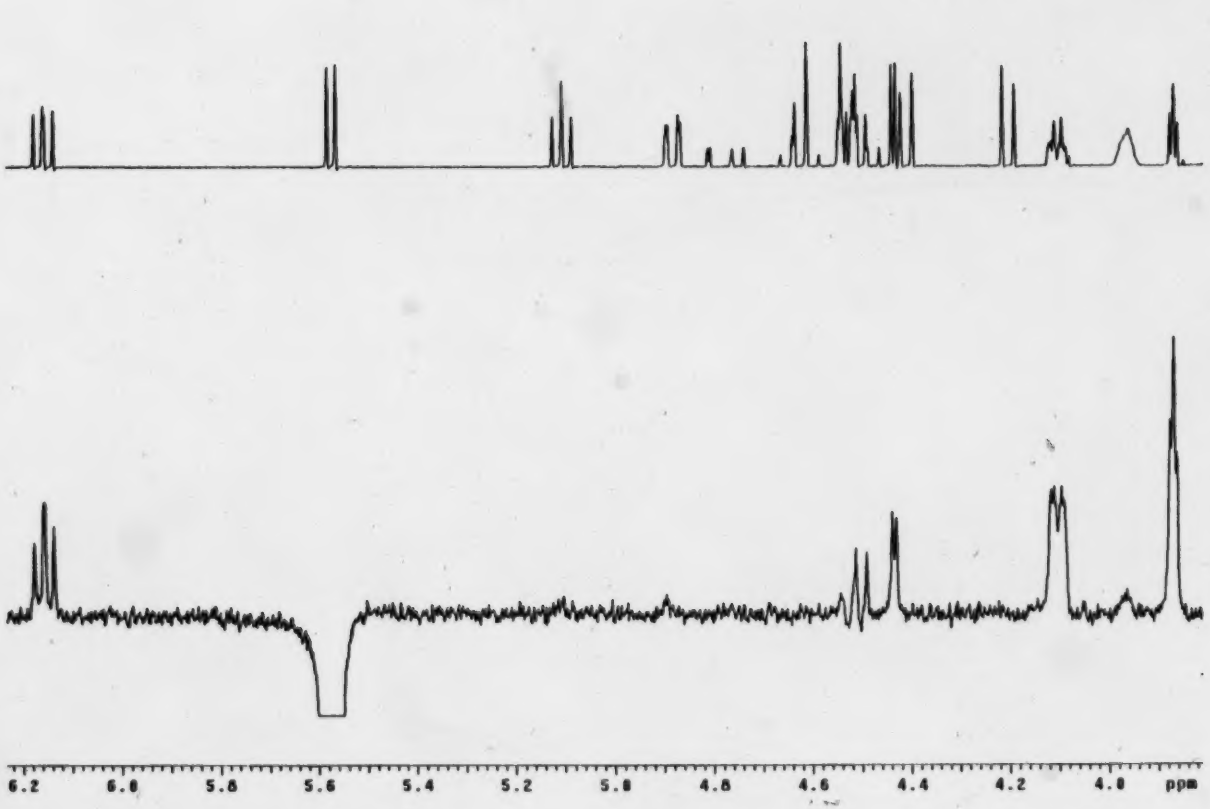


Fig 27. 1D TROESY of compound **27**.

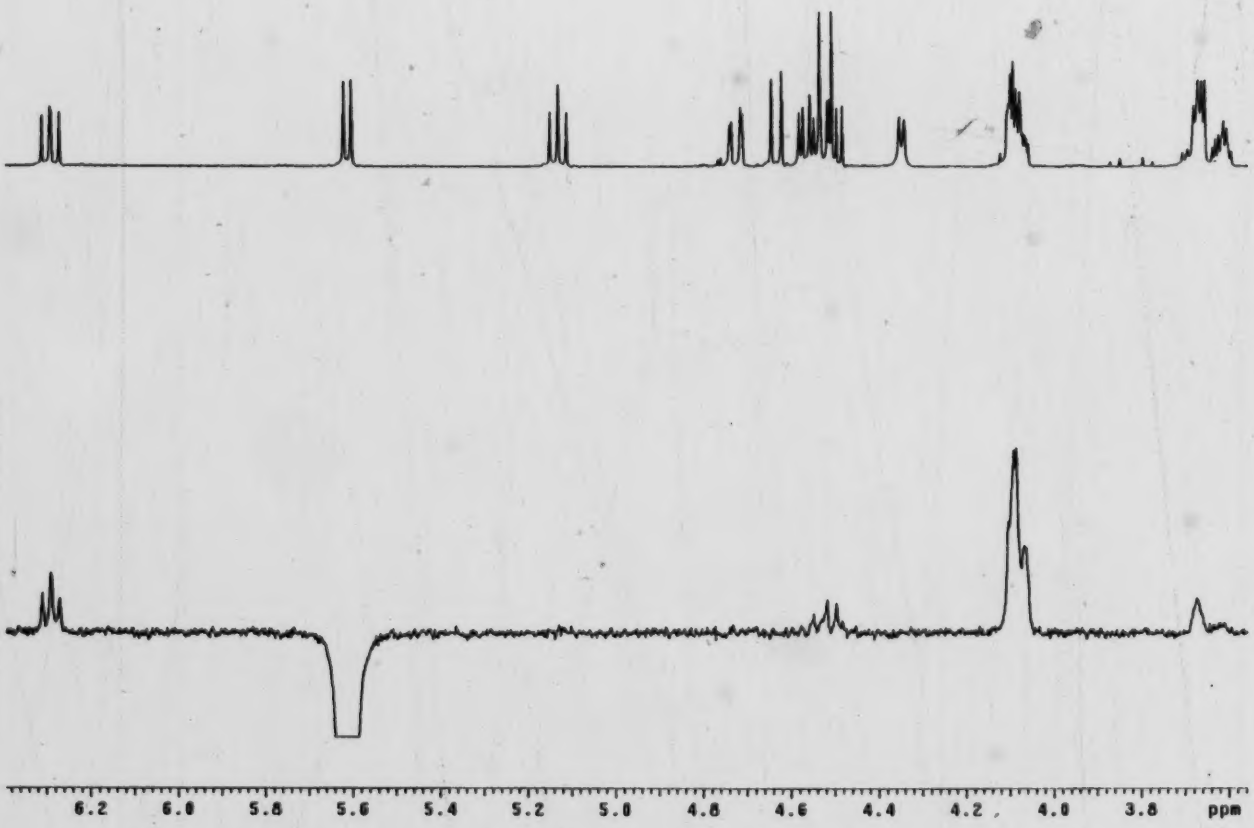


Fig 28. 1D TROESY of compound **26**.

disaccharide **27** occurs as a well resolved and isolated triplet δ 3.30 ppm (Fig 27). In NOE experiments (1D TROESY) an unambiguous NOE correlates H-1′ with H-2 indicating a short H-1′ to H-2 distance, typical of that seen between anomeric and aglyconic protons. The identification of the 1,3-linked disaccharide **26** was more complicated since the H-5′ and H-3 resonances overlap. In both disaccharides H-3′ and H-5′ exhibit strong NOEs from H-1′ due to their 1,3 diaxial relationships with H-1′ (Fig 28). The 1D TROESY for disaccharide **26** shows a weak NOE to H-2 but a significant NOE to H-3, that is seen as a partially resolved multiplet overlapped by the H-5′ multiplet. It was therefore concluded that the disaccharides obtained as an almost 1:1 mixture (48 to 52 %) were respectively the β1,2 (**27**) and β1,3 (**26**) linked disaccharides. This implied therefore, that the protection of the hydroxyl group at the 2 position was important in order to avoid the consumption of valuable donor. Coupling of the glucosamine imidate donor **25** with the tribenzylated lyxose acceptor **21** (Fig 29), was performed using TMSOTf as catalyst to give the disaccharide **28** in 87% yield.

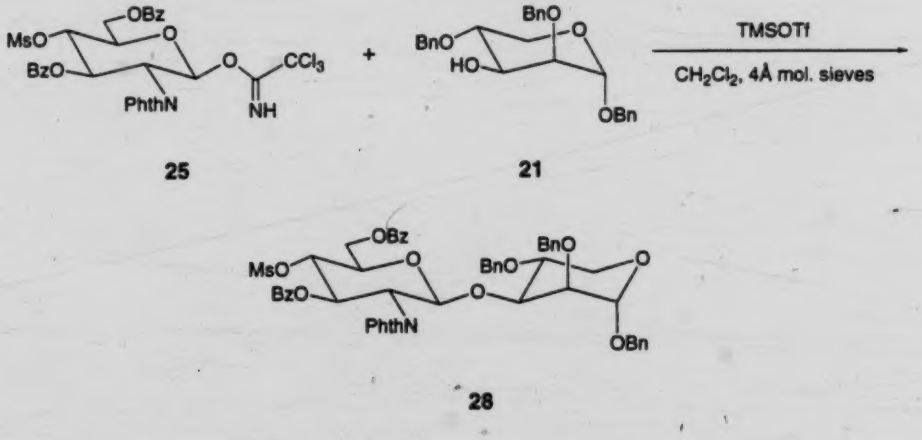
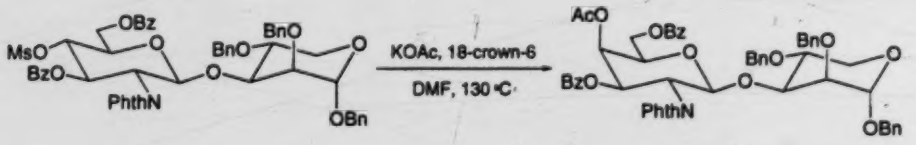


Fig 29. Synthesis of the glucosamine-lyxose disaccharide **28**.

Having assembled the disaccharide, the remaining transformation prior to deprotection was the nucleophilic displacement at C-4′. Published reports suggested two

methods: sodium acetate in water which yields the final product as the sugar with the hydroxyl group, or potassium acetate in DMF with the addition of crown ether to enhance the nucelophilicity of the anion.

The first method was not applicable because of the sensitivity to base of some of the groups present in the disaccharide. The crown ether method was chosen and due to the hygroscopic character of the potassium acetate, the reaction was carried out using sodium acetate. The yields were expected to be lower because the stability constant of the sodium ion-18-crown-6[84] is around 100 times smaller than that for the potassium complex. The possibility of using another crown ether to complex alkaline ions such as the 15-crown-5 was discarded because the stability constants of the sodium and potassium complexes are very close in value, but definitively lower than those of the 18-crown-6 ether. After 24 h the yields of compound **29** varied between 27-30% and showed little improvement when more crown ether was added. When potassium acetate was used, the reaction with disaccharide **29** (Fig 30) required 24 h to proceed in a 60%



28 **29**

Fig 30. Nucleophilic displacement at C-4' of disaccharide **28**.

yield and the unreacted starting material **28** could be recovered by chromatography. Previous studies done by Bundle et al.[74], reported the nucleophilic displacement on monosaccharides to proceed in 14 h with a 70 % yield. However, with the glucosamine-lyxose disaccharide **28** the displacement required longer reaction times and proceeded in 8-10% lower yields. In order to maximize overall yields, *t*-butyl glycoside **9** was first

converted to the *t*-butyl galactoside **30** (Fig 31) and then treated with TFA to give 4-*O*-acetyl-3,6-di-*O*-benzoyl-2-deoxy-2-phthalimido-α/β-D-galactopyranose (**31**)

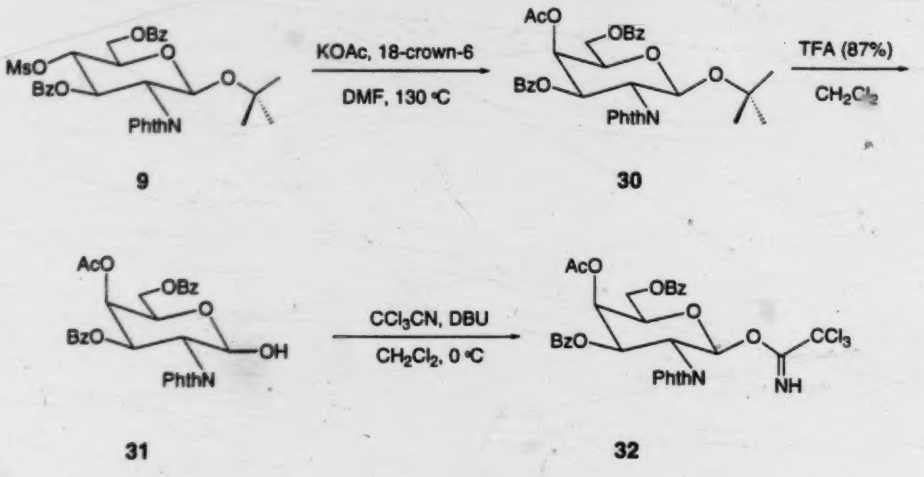


Fig 31. Synthesis of the galactosamine imidate **32**.

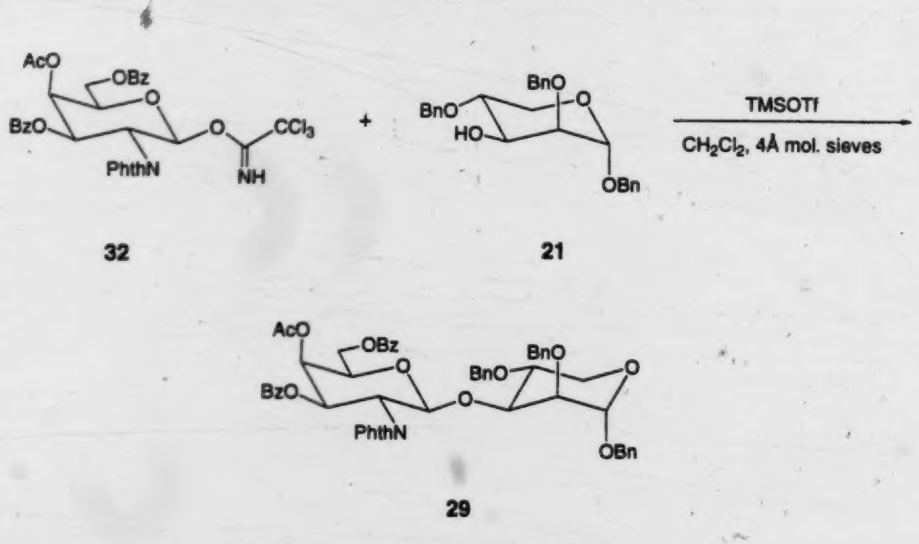


Fig 32. Synthesis of disaccharide **29** from compound **32**.

as a 4:1 β/α mixture. The galactosamine imidate **32** was obtained in 85% yield under the same conditions as those reported for compound **25.** The glycosidation of compound **32** with the tribenzylated acceptor **21** (Fig 32), gave the galactosamine-lyxose disaccharide 29 in 87% yield.

Removal of the acyl and amino protecting groups was achieved by treatment with hydrazine in ethanol (Fig 33)[85]. The 2′-amino-2′-deoxy disaccharide **33** was later used for the introduction of C-13 labeled acetate at the amino functionality. When crude **33** was acetylated with acetic anhydride in pyridine and chromatographed, the peracetylated disaccharide **34** was obtained in 81% yield. NMR studies of **34** showed coupling

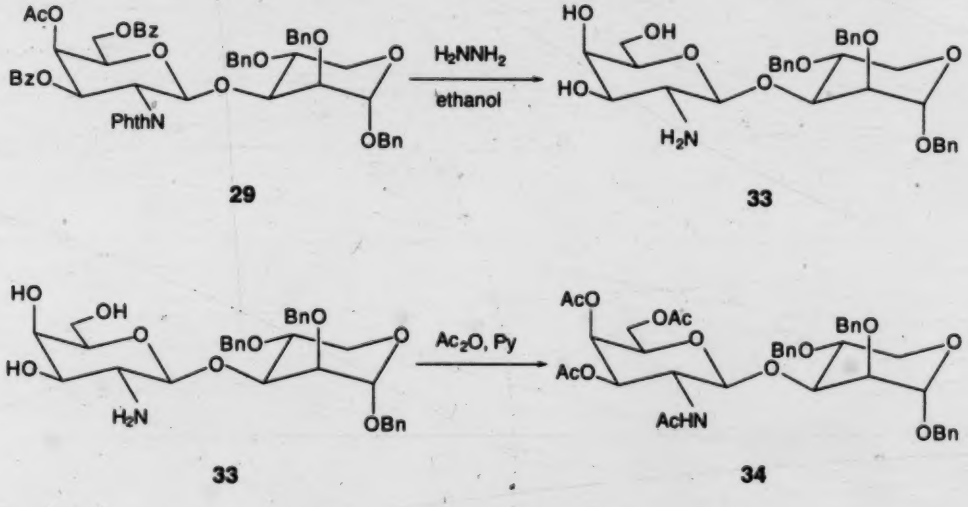
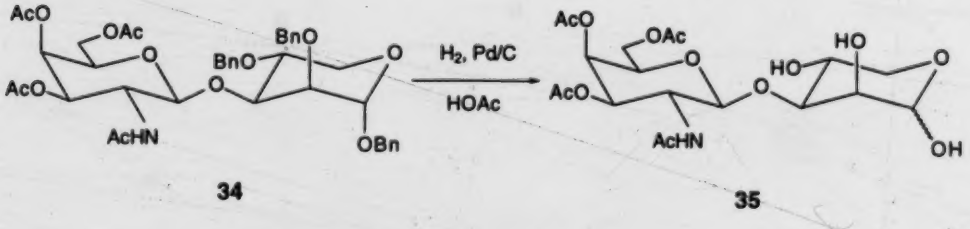


Fig 33. Removal of the protecting groups.

constants inconsistent with the expected $^4C_1$ conformation for the lyxose ring since $J_{1,2}$ = 7.0 Hz and $J_{4,5a}$ = 2.8 Hz with a C-H coupling constant for H-1 of 162.7 Hz a value more in agreement with an axial anomeric hydrogen atom. Minimization of the structures with the constant-valence force field (CVFF)[86] and the Newton-Raphson algorithm[87] showed the $^1C_4$ conformation to be 3.5 Kcal/mol more stable than the $^4C_1$

chair. Adoption of this chair form would explain the unusual coupling constants found in the ^{1}H NMR spectrum.

Hydrogenolysis of the benzyl groups gave the reducing disaccharide **35** (Fig 34). which was expected to be a mixture of anomers but appeared to be almost exclusively a single isomer. Although the spectral data were broadly consistent with the structure of **35** ambiguity existed for the anomeric proton. Its chemical shift was closer to that of a β isomer but had a $J_{1,2}$ coupling constant closer to that of an α isomer. Similarly the heteronuclear C,H coupling constant was in-between the usual values expected for an α and a β anomer. It seems likely that the pyranose ring is distorted or samples both $^1C_4$ and $^4C_1$ chair forms.



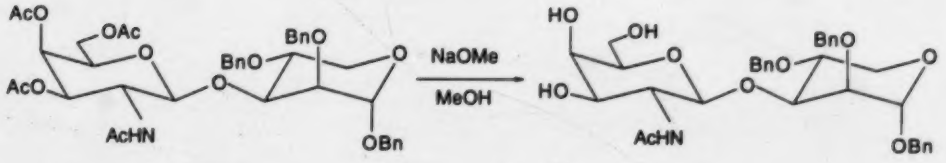
34 **35**

Fig 34. Removal of the benzyl groups.

2.1 Introduction of the ^{13}C label

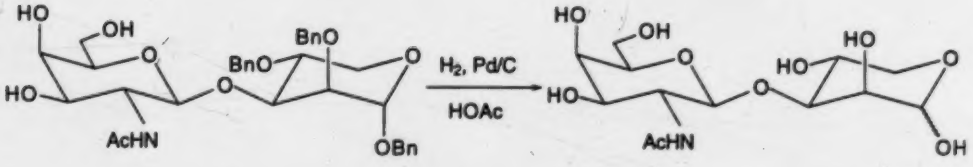
The central element of the synthetic scheme was the Kiliani-Fisher reaction between the disaccharide **35** and ^{13}C labeled potassium cyanide[66]. The procedure required a solution of the sugar in the minimum volume of water. This step could not be achieved, since the partially acetylated **35** proved to be only slightly soluble in water. The addition of a methanolic solution of the sugar to an aqueous solution of the potassium cyanide was not feasible due to the difficulties in controlling the pH of the reaction mixture. The most direct path proved to be the reaction on the fully deprotected disaccharide **37** obtained by transesterification of **34** (Fig 35) followed by palladium catalyzed

hydrogenolysis of the tribenzylated compound **36** to give the galactosyl-lyxose disaccharide **37** as a 4:1 α/β mixture (Fig 36).



34 **36**

Fig 35. Transesterification reaction of compound **34**.



36 **37**

Fig 36. Hydrogenolysis of compound **36**.

The general protocol for cyanohydrin formation requires a different ratio of sugar:cyanide and the yields are variable depending on the structure of the parent aldose[66]. For instance cyanohydrin formation from pentoses requires a three fold excess of cyanide because of their capability to form pyranose rings. Another important feature of this reaction is its dependence on pH, since a pH below 7 inhibits cyanohydrin formation, while a pH higher than 8.0 promotes hydrolysis of the cyanohydrin[88]. In the optimized procedure, the sugar is dissolved in water and added slowly to a cyanide solution whose pH is lowered to 7.2-7.5 by addition of 3M acetic acid. After 20 minutes the reaction mixture is acidified to pH 4.7 to eliminate the excess of cyanide by bubbling nitrogen through the solution to degas HCN. The flowthrough gases pass into a three

trap system containing methanolic potassium hydroxide, to recover the unused, labeled cyanide. The cyanohydrin is then reduced by hydrogenation at pH 4.3 using $Pd/BaSO_4$ catalyst. Under these conditions the reduction stops at the imine stage and the imine spontaneously hydrolyzes to the corresponding aldehyde.

Reaction on the galactosamine-lyxose disaccharide **37** (Fig 37) using a three fold excess of cyanide afforded no product most probably because the relative concentrations

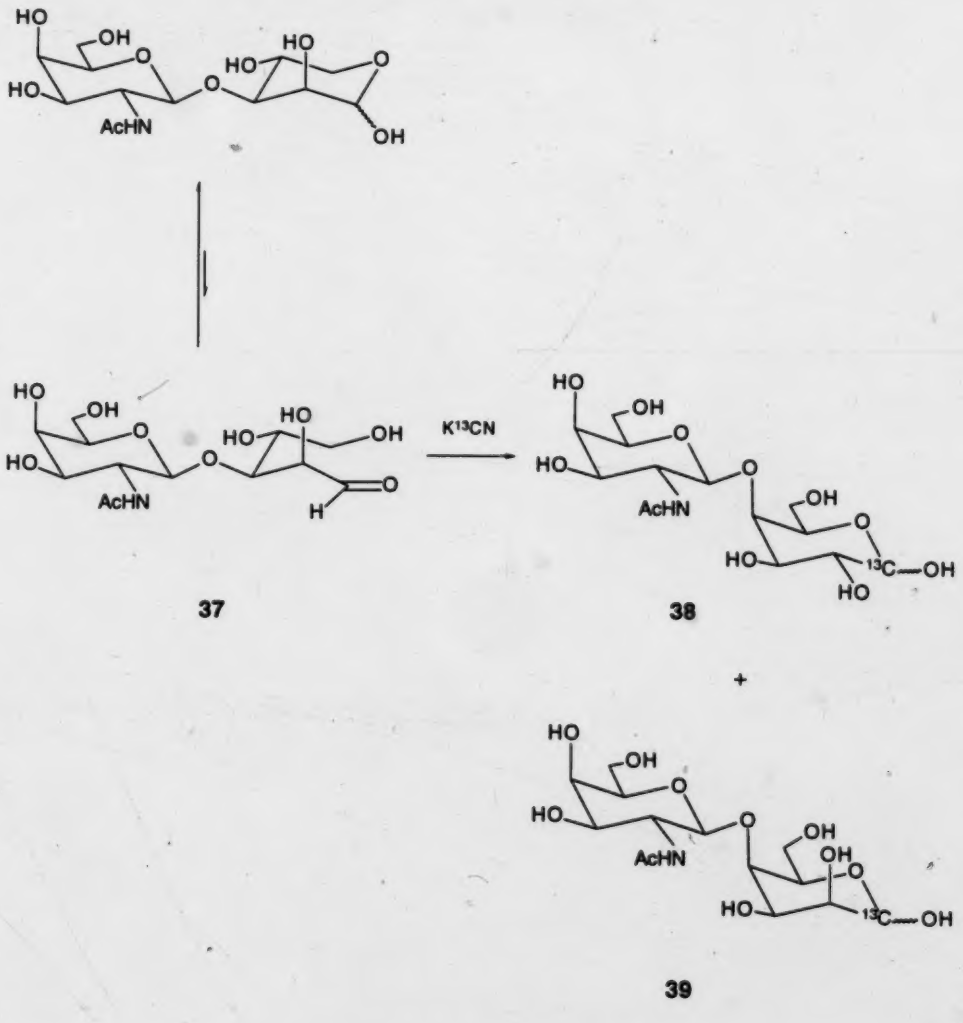


Fig 37. Cyanohydrin reaction.

of reagents were lower since the volume of water required to dissolve the disaccharide was larger than that needed for the monosaccharide. By increasing the cyanide:sugar ratio to 6:1 the yields improved to around 30%. Since the desired βGalNAc(1→4)α/βGal disaccharide was, on average, only 55% of the product formed, the reaction conditions had to be changed in order to improve the yield. This was accomplished by keeping the same ratio of cyanide:sugar, and the disaccharide was added portionwise as a solid, directly into the cyanide solution. The pH was controlled and adjusted when necessary after each addition. This modification in the technique allowed to increase the overall yield of the galactosaminyl-hexose to as much as 80 %.

Information obtained through ^{13}C NMR data allowed the estimation of the percentage of each diastereomer (**38** and **39**) and in addition, the ratio of the α/β anomers in the crude product. The following distribution was observed by ^{13}C NMR (Fig 38):

δ 97.40	β-D-GalNAc-(1→4)-**β-D-Gal**: 39%
δ 95.45	β-D-GalNAc-(1→4)-**α-D-Gal**: 21%
δ 95.11	β-D-GalNAc-(1→4)-**α-D-Tal**: 24%
δ 93.23	β-D-GalNAc-(1→4)-**β-D-Tal**: 16%

The ratio of galactose:talose in the Kiliani-Fisher reaction reported for monosaccharides is 1.3:1 and differs from the product distribution that would be predicted by Cram's rule. It is reported in the literature that some cyanohydrin preparations follow this rule while others do not, and the underlying cause is not known. When the Kiliani-Fisher reaction was performed on the galactosaminyl-lyxose disaccharide **37**, the ratio of the C-2 diastereomers **38** and **39** varied from 3:2 to almost exclusively galactose formation. The trend observed was that for low yield reactions, the percentage of the galactosamine-galactose disaccharide **38** was largest. In those cases when the reaction proceeded with the highest yield, the diastereomers **38** and **39** were

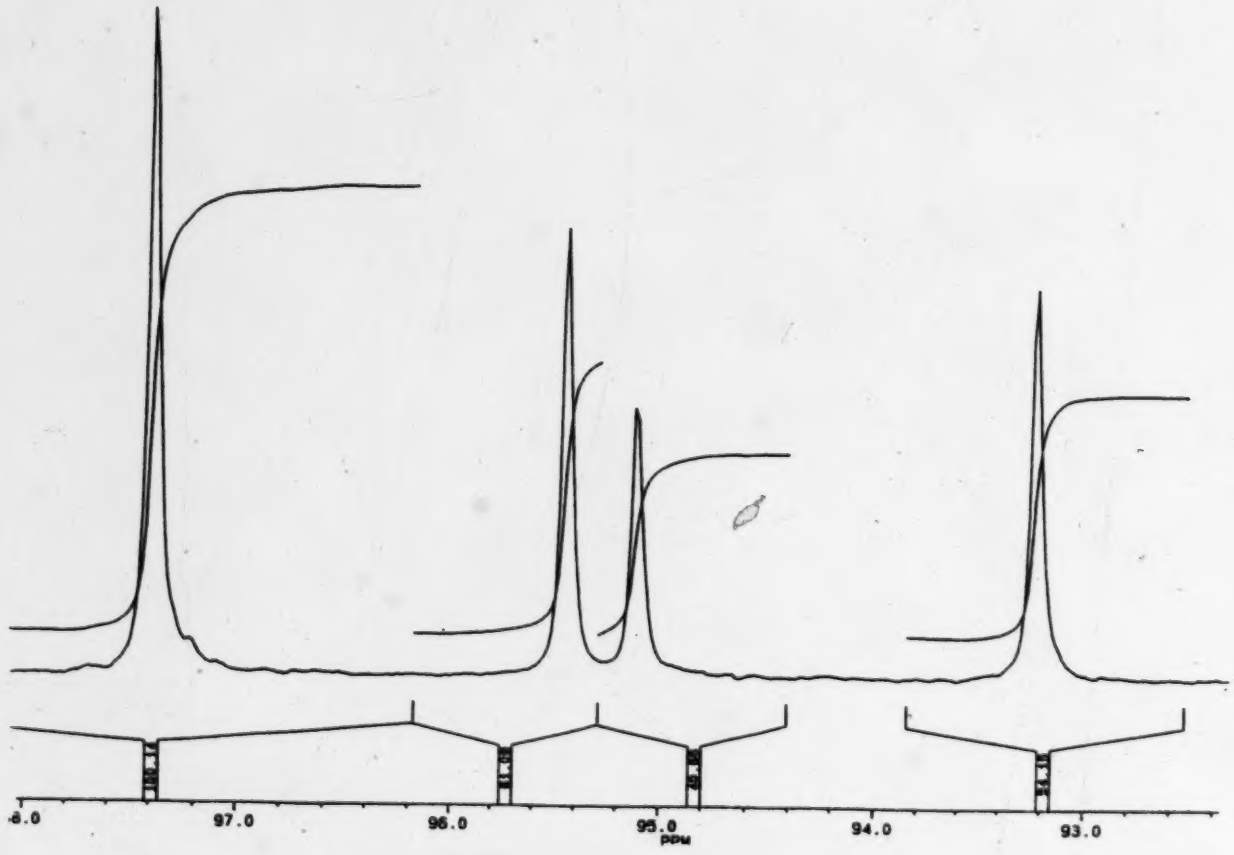


Fig 38. ^{13}C NMR of the crude labeled mixture.

obtained in a 3:2 ratio. The ^1H NMR data and a semiselective coupled heteronuclear correlation experiments (HMQC) of the crude product allowed the assignment of all four anomeric protons with their respective coupling constants:

δ 5.22	dd, $J_{1,2}$ 3.8 Hz, $^1J_{^1H,^{13}C}$ 169.7 Hz	**α-D-Gal**
δ 5.21	dd, $J_{1,2}$ 1.7 Hz, $^1J_{^1H,^{13}C}$ 171.6 Hz	**α-D-Tal**
δ 4.75	d, $^1J_{^1H,^{13}C}$ 164.7 Hz	**β-D-Tal**
δ 4.56	dd, $J_{1,2}$ 7.9 Hz, $^1J_{^1H,^{13}C}$ 161.5 Hz	**β-D-Gal**

At this point, it was necessary to purify and isolate the mixture of C-2 diastereomers (**38** and **39**) and starting material (**37**). Preferential complexation of the monosaccharide talose to a Ca^{2+} column has been shown to be an effective way of resolving mixtures of galactose and talose monosaccharides (Fig 39)[69,70]. When the mixture is eluted through a calcium loaded cationic resin, talose with an axial-equatorial-axial pattern of hydroxyl groups is retained while galactose moves with the solvent (water).

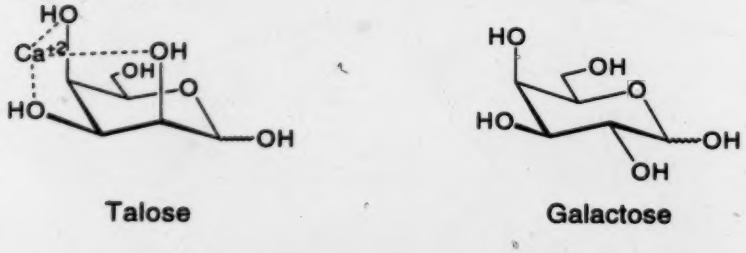


Fig 39. Isolation of monosaccharides in a calcium loaded resin.

When the same principle was applied to the mixture of galactosamine-galactose (**38**) and galactosamine-talose (**39**) disaccharides, the whole sample eluted without being resolved. The separation problem was overcome by acetylating the mixture to give the peracetylated derivatives **40** and **41** (Fig 40) which were isolated by chromatography. The first compound that eluted from the column was identified as the peracetylated βGalNAc(1→4)α/βGal disaccharide **40** from which the βGalNAc(1→4)βGal could be

isolated by taking the first cuts. The unreacted peracetylated galactosyl-lyxose disaccharide eluted very close to this compound, followed by the peracetylated galactosyl-talose derivative **41**. The presence of the ^{13}C isotope at the C-1 of the galactose residue resulted in an atypical appearance in the ^{1}H NMR spectrum for **40**. Since the H-1 proton experienced a large heteronuclear coupling constant $^{1}J_{H,^{13}C} \approx$ 166.7 Hz its resonance appears as a well separated doublet of doublets at δ 5.66 with $J_{1,2}$ 8.4 Hz. The ^{1}H NMR spectrum also showed H-2 as a doublet of doublet of doublets due to the couplings to H-1 and H-3 as well as the two bond coupling to the ^{13}C-1, while H-3 and H-5 showed ^{3}J couplings to ^{13}C-1 besides the usually expected couplings to their vicinal hydrogens[89].

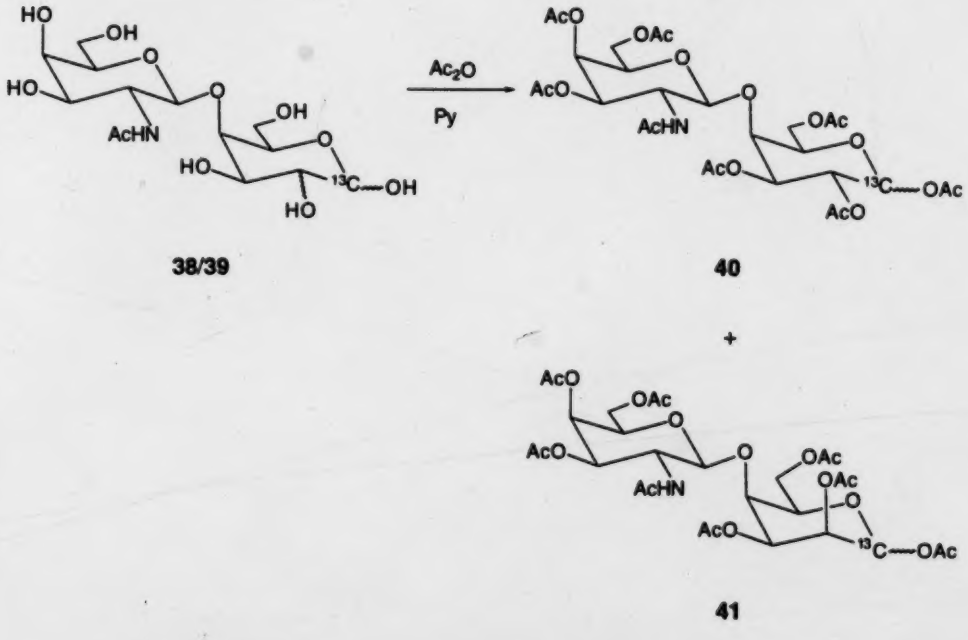


Fig 40. Isolation of the epimers **38** and **39** by acetylation.

In order perform the NOE studies between the protein and the sugar, it is necessary to attach a protein or a carrier to the carbohydrate. Generally, the coupling of the two

parts is done using a linker such as an allyl residue. For the synthesis of an β-allyl glycoside, it was necessary to consider first the activator, solvent and catalytic conditions to be used.

Because glycosyl trichloroacetimidates react under mild conditions with predictable stereochemistry, it was decided to use this approach for the synthesis of the allyl glycoside **44**[90]. Prior to the formation of the imidate it was necessary to remove the anomeric acetyl group which was accomplished by reaction of the peracetylated GalNAc-Gal disaccharide **40** with hydrazine acetate in dry DMF (Fig 41). Crude **42**

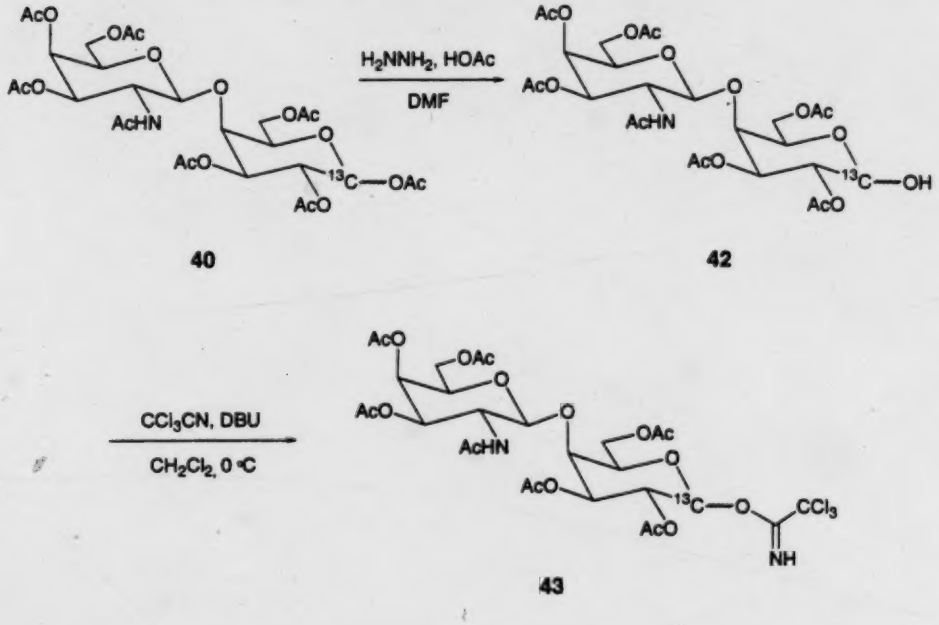


Fig 41. Synthesis of the imidate **43**.

was used directly for the synthesis of the imidate **43** following the procedure reported for the synthesis of the glucosamine imidate **25**. The ^{1}H NMR of the imidate indicated the almost exclusive formation of the α imidate with H-1 appearing as a doublet of doublets d at δ 6.48 with coupling constants $J_{1,2}$ and $^1J_{^1H,^{13}C}$ of 3.7 and 179.5 Hz,

respectively. At this point, for the formation of the allyl glycoside **44** it was important to evaluate the solvent and the catalyst to be used in order to obtain the target β allyl glycoside. Reactions catalyzed by boron trifluoride etherate in dichloromethane have been reported to proceed with inversion of configuration even in sugars with non participating groups at C-2, whereas, the ones catalyzed by TMSTf give products with retention, or inversion depending on the temperature and the solvent used[90]. The fact that the imidate **43** had an α configuration with a participating group at C-2, prompted the choice of dichloromethane as solvent, with trimethylsilyl triflate as catalyst, and a

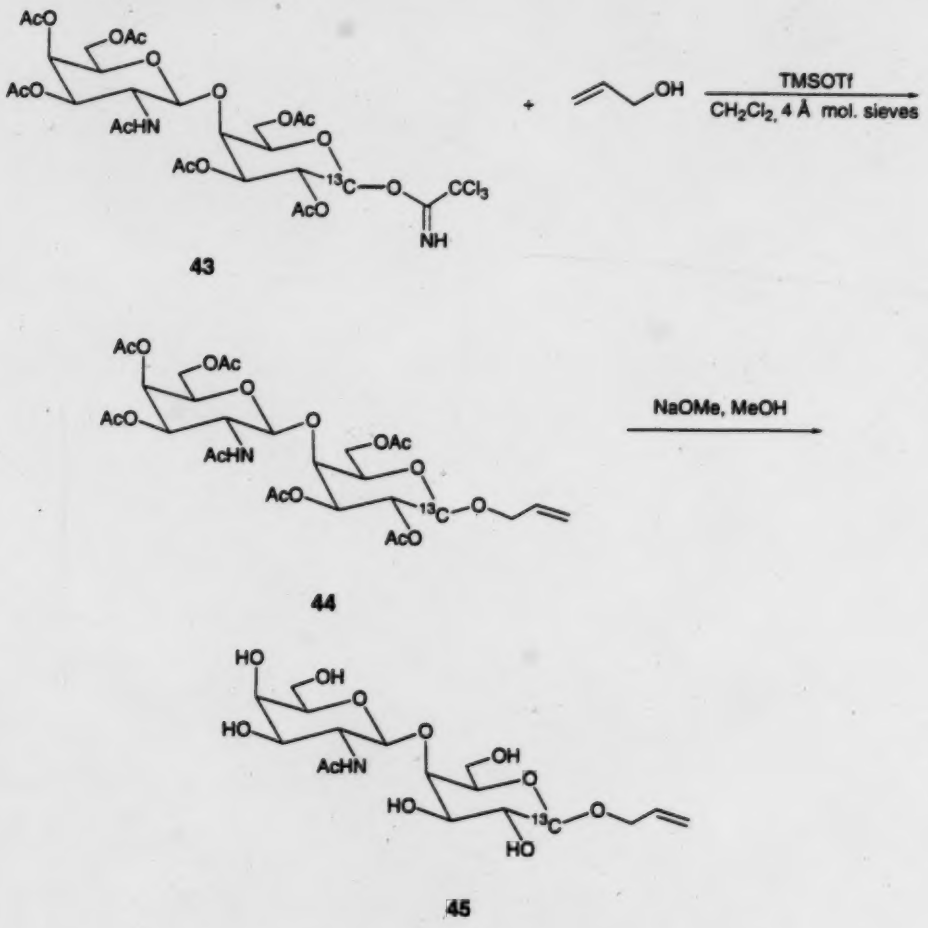


Fig 42. Synthesis of the allyl glycoside **45**.

temperature of -15 °C up to -10 °C as the best conditions to obtain the β-linked allyl glycoside **44** (Fig 42). ^1H NMR of the main reaction product confirmed the formation of the β glycoside constants $^1J_{1_H,13_C}$ 158.6 Hz and $J_{1,2}$ 7. **44** with a resonance signal for H-1 at δ 4.48 and coupling constants $^1J_{1_H,13_C}$ and $J_{1,2}$ of 167.1 and 8.3 Hz, respectively. Transesterification gave the target glycoside **45** whose β-configuration was confirmed by the ^1H NMR signal for H-1 at δ 4.24 with coupling 9 Hz.

2.2 Introduction of the Label at the Galactosamine Residue

The introduction of the label at the galactosamine residue was achieved by acetylation of compound **33** with labeled acetyl chloride in dry dichloromethane (Fig 43). ^1H NMR spectral data of the crude product showed the proton signal of the methyl group at δ 1.96 as a doublet of doublets with a $^1J_{1_H,13_C}$ of 128.8 Hz and a $^2J_{1_H,13_C}$ of 6.2 Hz while in the ^{13}C NMR spectrum both the signals of the carbonyl and the methyl carbons appeared as doublets with a $^1J_{13_C,13_C}$ of 54.49 Hz. The ESMS spectrum showed

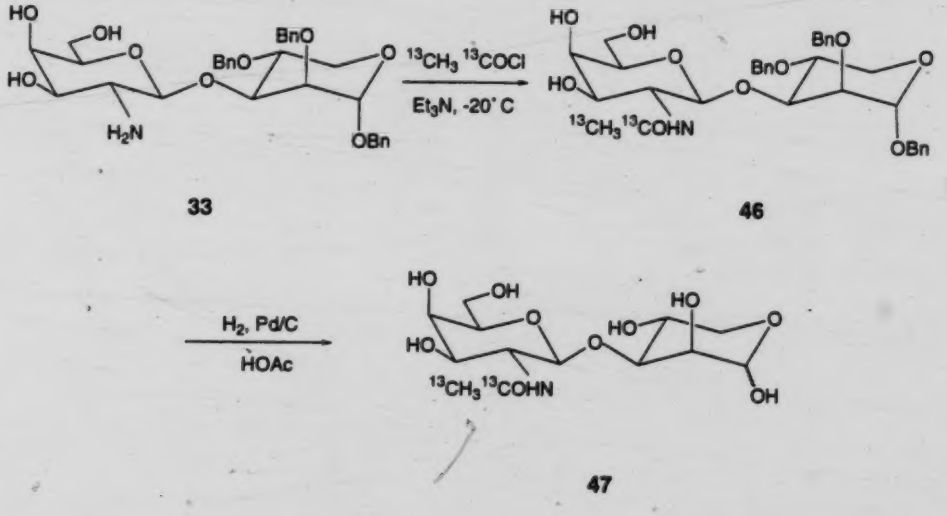
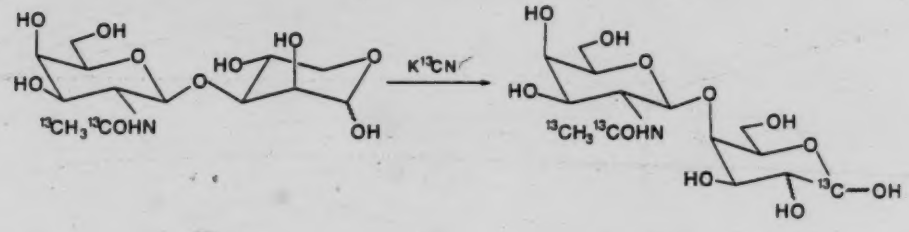


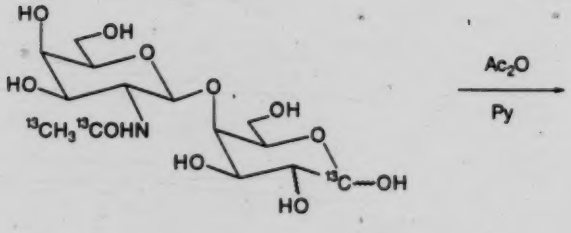
Fig 43. Introduction of the labeled acetate at the amino functionality.

an expected peak pattern two mass units higher than that from compound **36**, in accordance with the introduction of two ^{13}C enriched atoms in the molecule. The removal of the benzyl groups from **46** was done by hydrogenolysis using the same procedure reported for compound **37**. From this point onwards, the isolation of



47 **48/49**

Fig 44. Cyanohydrin reaction on compound **47**.



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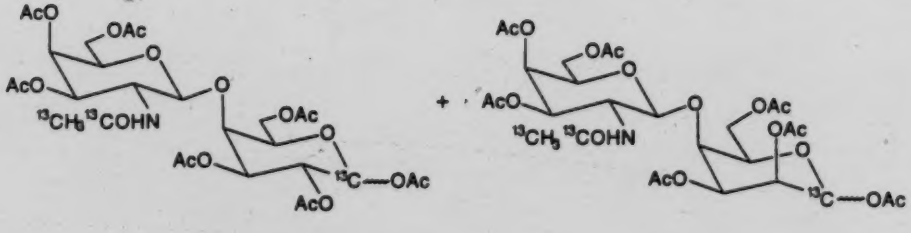


50 **51**

Fig 45. Isolation of the C-2 epimers **48** and **49**.

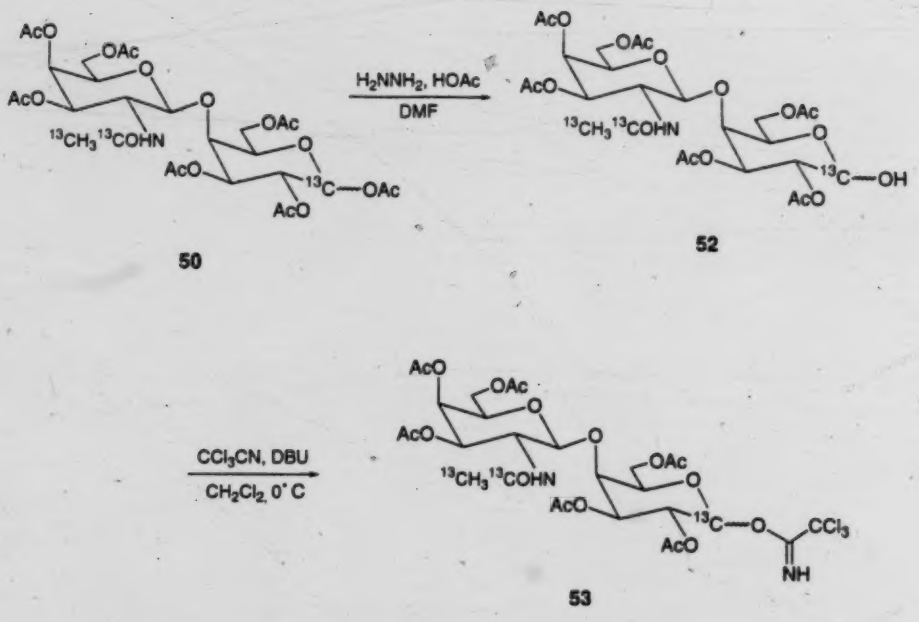


Fig 46. Synthesis of the tri-labeled imidate **53**.

compounds **50** and **51** by peracetylation/chromatography of the mixture was done under the conditions reported for compounds **38, 39, 40** and **41** (Fig 44 and 45). The procedures followed for the formation of the imidate **53** (Fig 46) and for the coupling of the allyl residue to give the trilabeled allyl glycoside **54**, were similar to the ones described for the monolabeled disaccharide (Fig 47). The last step was the removal of the acetates to give compound **55**.

^1H NMR and ^{13}C NMR spectral data of the tri-labeled compounds were comparable to those of the monolabeled ones, with exception of the resonance signals derived from the labeled acetyl residue. High resolution ESMS of all of them exhibited molecular ion monolabeled peaks and fragmentation peak patterns two units higher than the corresponding disaccharides.

In all the ^1H NMR spectra of the compounds containing the ^{13}C labeled acetyl group, the presence of a small doublet at δ 1.97 with a $^1J_{^{13}C,^{13}C} \approx 128$ Hz was

observed. This was attributed to the presence of a small percentage of unlabeled carbonyl carbon. This could be confirmed through mass spectrometry, that showed a higher abundance than expected of the $[(M-1)+Na]^+$ peak.

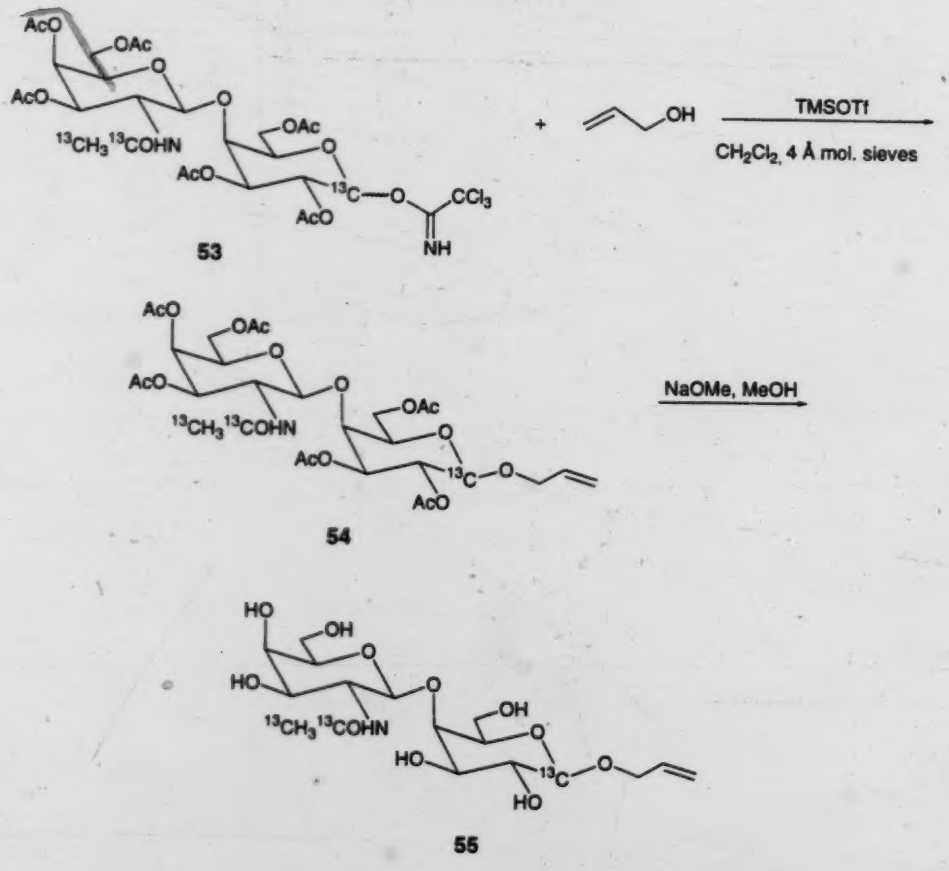


Fig 47. Synthesis of the tri-labeled allyl glycoside **55**.

As mentioned before, the objective of the research was to carry out NMR studies of the PAK pili protein bound to the labeled sugar disaccharide. The experiments are expected to provide information about the pilus amino acid residues that come into contact with the disaccharide epitope. In order to perform such NMR studies it is

necessary to covalently attach the disaccharide to a protein residue or other carrier molecule. This may be done via the allyl residue at the anomeric center (Fig 48)[91].

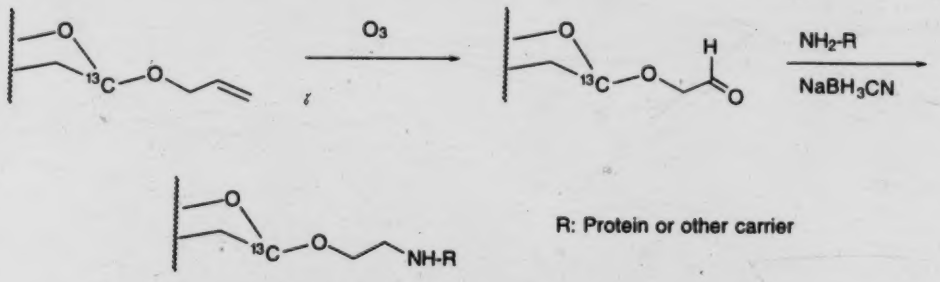


Fig 48. Covalent attachment to protein via the allyl group.

Preparation of an oligovalent saccharide-carrier conjugate amplifies the functional affinity of the complex with PAK pili. Since the complex is longer lived there is a greater opportunity to observe chemical shift changes and possibly NOEs between the saccharide and the pilus protein. These studies, which will be performed as part of on-going work, should provide information on the orientation of the saccharide towards the binding site of the adhesin.

3 Experimental Procedures

3.1 General Methods:

Microanalysis were carried out by the analytical services at this department on a Carlo Erba 1108 analyzer. All the samples submitted for elemental analysis were dried overnight under vacuum with phosphorous pentoxide at 56 °C (refluxing acetone). Optical rotations were measured with a Perkin Elmer 241 polarimeter at 22±2 °C. Fast atom bombardment mass spectra (FAB) were recorded on samples suspended in a Cleland matrix (5:1 mixture of dithiothreitol:dithioerytritol) with xenon as the bombarding gas in a AEIMS-9 spectrometer. The mass spectra performed by positive mode electrospray ionization were done on a Micromass ZabSpec Hybrid Sector-TOF using either a 1% solution of acetic acid in 1:1 water:methanol or 1:3 toluene:methanol as the liquid carrier and nitrogen as the spray pneumatic aid. Analytical TLC was performed on Silicagel 60-F254 (E. Merck, Darmstadt) with detection by quenching of fluorescence and/or charring with sulfuric acid. All commercial reagents were used as supplied and chromatography solvents were distilled prior to use. Solvents for anhydrous reactions were dried according to literature procedures[92].

Toluene was distilled over sodium; pyridine and dichloromethane were distilled over calcium hydride; *N,N*-dimethylformamide was distilled under reduced pressure from calcium hydride; methanol was distilled from magnesium turnings and a catalytic amount of iodine; deionized water was obtained from a Millipore Milli-Q plus apparatus. Carbon-13 labeled reagents were supplied by Cambridge Isotope Laboratories. Column chromatography was performed on Silica gel 60 (E. Merck 32-63 micron, 60A, Darmstadt) or on Iatrobeads purchased from Iatron Laboratories, Inc. (Tokyo, Japan) as indicated. All the resins used were washed with methanol, water, the desired ionic species and finally washed with deionized water prior to use.

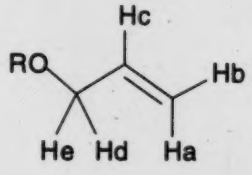
Computational results obtained using software programs from MSI of San Diego were done with the Discover ® program, using the CVFF forcefield[86]. Molecular

structures were minimized using the Conjugate Gradient method of Polak and refined by the Newton-Raphson technique[87].

^{1}H NMR were recorded at 300 MHz (Bruker AM-300), 360 MHz (Bruker WM-360), 500 MHz (Unity 500-Varian) or 600 MHz (Inova 600-Varian) as indicated, using acetone as the internal reference (δ 2.225) for solutions in D_2O, or the residual solvent proton peaks for solutions in $CDCl_3$ (δ 7.24) or CD_3OD (δ 3.30). ^{13}C NMR were recorded at 300 MHz (Bruker AM 300) with acetone as internal reference (δ 31.0) for solutions in D_2O, or the ^{13}C resonance of solvent $CDCl_3$ (δ 77.0) or CD_3OD (δ49.0). Spectra recorded at 500 and 600 MHz employed temperature-controlled conditions at 30 ±0.1 °C, using VNMR software. All GCOSY experiments[93] were recorded with a single transient per t_1 increment and a 1.2 s relaxation delay. Both gradients were rectangular in shape, applied in the z direction, of strength 0.6 G/cm and 10 ms duration, with gradient rise and fall times of 10 μs. The RF pulses were calibrated 90° pulses of 7.5 μs duration. Two dimensional spectra were recorded nonspinning in absolute-value mode with a sweep width of 2500 Hz in both dimensions and 4K data points in F_2 (zero-filled to 8K) and 1K (zero-filled to 2K) data points in F_1, resulting in digital resolutions of 0.6 and 2.4 Hz/pt, respectively. Prior to Fourier transformation, the FIDs were multiplied by unshifted sine-bell square functions of width $t_2/2$ and $t_1/2$, respectively. One dimensional T-ROESY spectra[94,95] were recorded with a 185 ms eburp-1 excitation pulse and a sweep width of 2500 Hz. The mixing time was 150 ms at a power level of 2.1 kHz and 128 scans were accumulated. Line broadening of 0.5 Hz was applied prior to Fourier transformation of the 16K (zero-filled to 32K) data points. Proton coupled HMQC spectra were acquired with quadrature detection in F_1 and a total of 512t_1 increments of 320 scans each were recorded for a spectral width in F_2 of 7800 Hz and 1500 Hz in F_1[96]. Data were processed to give after zero-filling a matrix (t_1 x t_2) of 512 x 8192 points, and following resolution enhancement as previously described, double transformation gave power spectra. Fixed delays of τ_1 = 3.4 ms and τ_2 = 1.7 ms

were employed to select all multiplicities, and a recycle delay of ca. 1 s (~ one proton T_1) was used. ^{13}C NMR shifts assignments are tentative unless determined by two dimensional C/H correlation experiments and were assigned based on comparison with related compounds.

All literature compounds had spectral data and elemental analysis consistent with the reported values. Protons of the allyl group present in the compounds described in the thesis were designated Ha, Hb, Hc, Hd, and He as defined below.



3.2 Synthesis

3,6-di-O-benzoyl-2-deoxy-4-mesyl-2-phthalimido-β-D-glucopyranose **(10)**[74]. To a solution of compound **9** (2.610 g, 4 mmol) in dichloromethane (9 ml) was added trifluoroacetic acid (18.2 ml) and the solution was left stirring at room temperature for 45 min. After concentration, the selectively protected glucopyranose compound **10** was obtained as a pale yellow foam (2.271 g, 96 %), 0.40 (pentane:ethyl acetate 3:1). ^1H NMR (360 MHz, $CDCl_3$): δ_H 8.15-7.30 (m, 14H, Ph), 6.25 (dd, 1H, $J_{2,3}$ 10.7 Hz, $J_{3,4}$ 9.1 Hz, H-3), 5.79 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 5.17 (t, 1H, $J_{4,5}$ 9.6 Hz, H-4), 4.78 (dd, 1H, $J_{5,6a}$ 1.8 Hz, $J_{6a,6b}$ 12.6 Hz, H-6a), 4.56 (dd, 1H, $J_{5,6b}$ 4.0 Hz, H-6b), 4.46 (dd, 1H, H-2), 4.17 (ddd, 1H, H-5), 2.86 (s, 3H, SO_2CH_3).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 166.27, 165.28 (C=O), 134.37, 133.67, 133.32 (Ph methine), 131.40 (Ph quaternary), 130.00 (Ph methine), 129.69 (Ph quaternary), 128.58, 128.52, 123.77 (Ph methine), 92.83 (C-1), 74.85 (C-4), 72.33 (C-5), 70.43 (C-3), 56.29 (C-2), 38.94 (SO_2CH_3).

Anal. Calcd for $C_{29}H_{25}O_{11}NS$ (595.58): C, 58.48; H, 4.23; N, 2.35; S, 5.38. Found: C, 58.26; H, 4.04; N, 2.27; S, 5.39.

3,6-di-O-benzoyl-2-deoxy-4-mesyl-2-phthalimido-β-D-glucopyranosyl bromide (**11**). A solution of oxalyl bromide (385 ml, mmol) in dichloromethane (2 ml) was added dropwise to a stirred solution of **10** (940 mg, 1.58 mmol) and DMF (39 ml) in dichloromethane (5 ml). After 2 h the mixture was diluted with dichloromethane (8 ml) and poured into ice water. The organic layer was washed with cold water (3 x 20 ml), dried (sodium sulfate) and concentrated to give the glycosyl bromide **11** as a pale yellow foam containing small amounts of unreacted starting material, R_f 0.68 (pentane:ethyl acetate 3:2). ^{1}H NMR (360 MHz, $CDCl_3$): δ_H 8.20-7.30 (m, 14H, Ph), 6.50 (d, 1H, $J_{1,2}$ 9.6 Hz, H-1), (dd, 1H, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 9.3 Hz, H-3), 5.21 (t, 1H, $J_{4,5}$ ~9.9 Hz, H-4), 4.80 (m, 2H, H-2, H-6a), 4.58 (dd, 1H, $J_{5,6b}$ 4.3 Hz, $J_{6b,6a}$ 12.7 Hz, H-6b), 4.20 (ddd, 1H, $J_{5,6a}$ 2.0 Hz, H-5), 2.85 (s, 3H, $SO_2C\underline{H}_3$).

3,6-di-O-benzoyl-2-deoxy-4-mesyl-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**25**). Compound **10** (3.109 g, 4.16 mmol) was dissolved in dry dichloromethane under a nitrogen atmosphere and cooled at 0 °C. Trichloroacetonitrile (9 ml, 90 mmol) and DBU (360 µl) were added to the mixture. After stirring for 45 min at 0 °C the solution was concentrated and chromatographed (pentane:ethyl acetate 3:1) to give imidate **25** as a clear residue (2.705 g, 87%) R_f 0.47 (pentane:ethyl acetate 2:1). ^{1}H NMR (360 MHz, $CDCl_3$): δ_H 8.68 (s, 1H, C=N$\underline{H}$), 8.18-7.30 (m, 14H, Ph), 6.64 (d, 1H, $J_{1,2}$ 8.8 Hz, H-1), 6.30 (dd, 1H, $J_{2,3}$ 10.7 Hz, $J_{3,4}$ 9.0 Hz, H-3), 5.24 (dd, 1H, $J_{4,5}$ 9.8 Hz, H-4), 4.81 (dd, 1H, H-2), 4.79 (dd, 1H, $J_{5,6a}$ 2.1 Hz, $J_{6a,6b}$ 12.6 Hz, H-6a), 4.64 (dd, 1H, $J_{5,6b}$ 4.0 Hz, H-6b), 4.31 (ddd, 1H, H-5), 2.88 (s, 3H, $SO_2C\underline{H}_3$). ^{13}C NMR (300 MHz, $CDCl_3$): δ_C 95.00 (C-1).

ESMS m/z = 761 $[M+Na]^+$, ($C_{31}H_{25}O_{11}N_2SCl_3$ requires m/z = 738).

tert-Butyl 4-O-acetyl-3,6-di-O-benzoyl-2-deoxy-2-phthalimido-β-D-galactopyranoside **(30)**. Dicyclohexano-18-crown-6 (88 mg, 0.23 mmol) and dry potassium acetate (526 mg, 5.35 mmol) were added to a solution of compound **9** (1 g, 1.54 mmol) in dry DMF (12 ml). The suspension was stirred for 14 h at 130 °C, cooled, poured into ice-water and the mixture extracted with ethyl acetate. The combined extracts were washed with water, dried, concentrated and chromatographed (pentane:ethyl acetate step gradient from 1:5 to 1:3) to give glycoside **30** (660 mg, 70%) $[\alpha]_D$ +30.4° (*c* 1, CH_2Cl_2); literature:+21.9° (*c* 0.6, CH_2Cl_2;), R_f 0.43 (pentane:ethyl acetate 3:1). ^{1}H NMR (360 MHz, $CDCl_3$): δ_H 8.10-7.20 (m, 14H, Ph), 6.11 (dd, 1H, $J_{2,3}$ 11.4 Hz, $J_{3,4}$ 3.5 Hz, H-3), 5.75 (d, 1H, $J_{4,5}$ ~3.5 Hz, H-4), 5.55 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 4.75 (dd, 1H, H-2), 4.55 (dd, 1H, $J_{5,6a}$ 7.3 Hz, $J_{6a,6b}$ 11.1 Hz, H-6a), 4.18 (dd, 1H, $J_{5,6b}$ 5.8 Hz, H-6b), 4.30 (t, 1H, H-5), 2.17 (s, 3H, $OCOC\underline{H}_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 170.12, 166.08, 165.12 (C=O), 134.24, 133.34, 133.25 (Ph methine), 131.48 (Ph quaternary), 129.78, 129.65 (Ph methine), 129.07 (Ph quaternary), 128.61, 128.48, 123.52 (Ph methine), 93.66 (C-1), 76.83 ($\underline{C}(CH_3)_3$), 70.93 (C-5), 68.83 (C-3), 67.13 (C-4), 62.47 (C-6), 52.15 (C-2), 28.57 ($C(\underline{C}H_3)_3$), 20.73 ($OCO\underline{C}H_3$).

FABMS (cleland) m/z = 638 $[M+Na]^+$, ($C_{34}H_{33}O_{10}N$ requires m/z = 615).

4-O-acetyl-3,6-di-O-benzoyl-2-deoxy-2-phthalimido-β-D-galactopyranose **(31)**. *t*-Butyl glycoside **30** (2.05 g, 3.3 mmol) was dissolved in dichloromethane (7 ml) and trifluoroacetic acid (14 ml) was added. The solution was stirred for 45 min and concentrated to dryness to give the galactopyranose **31** (1.850 g, 99%) as a pale brown foam, R_f 0.32 (pentane:ethyl acetate 2:1). ^{1}H NMR (360 MHz, $CDCl_3$): δ_H 8.10-7.20 (m, 14H, Ph), 6.05 (dd, 1H, $J_{2,3}$ 11.4 Hz, $J_{3,4}$ 3.4 Hz, H-3), 5.78 (d, 1H, $J_{4,5}$ ~3.3

Hz, H-4), 5.72 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 4.72 (dd, 1H, H-2), 4.52 (dd, 1H, $J_{5,6a}$ 4.9 Hz, $J_{6a,6b}$ 11.4 Hz, H-6a), 4.40-4.30 (m, 2H, H-5, H-6b), 2.17 (s, 3H, OCOC$\underline{H}_3$).
^{13}C NMR (300 MHz, $CDCl_3$): δ_C 169.96, 166.17, 165.08 (C=O), 134.37, 133.43, 133.38 (Ph methine), 131.48 (Ph quaternary), 129.87, 129.65 (Ph methine), 128.91 (Ph quaternary), 128.51 (Ph methine), 93.16 (C-1), 71.41 (C-5), 68.64 (C-3), 67.05 (C-4), 62.16 (C-6), 53.20 (C-2), 20.65 (OCO$\underline{C}H_3$).
ESMS m/z = 598 $[M+K]^+$, 582 $[M+Na]^+$ ($C_{30}H_{25}O_{10}N$ requires m/z = 559).

4-O-acetyl-3,6-di-O-benzoyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl trichloroacetimidate (**32**). Compound **31** (1.870 g, 3.3 mmol) was dissolved in dichloromethane under a nitrogen atmosphere and cooled at 0 °C. Then trichloroacetonitrile (9 ml, 90 mmol) and DBU (275 µl) were added to the mixture. After stirring for 45 min at 0 °C the solution was concentrated and chromatographed (pentane:ethyl acetate 5:1) to give trichloroacetimidate **32** (1.972 g, 85 %) as a clear residue R_f 0.53 (pentane:ethyl acetate 3:1). ^{1}H NMR (360 MHz, $CDCl_3$): δ_H 8.65 (s, 1H, C=N$\underline{H}$), 8.10-7.25 (m, 14H, Ph), 6.75 (d, 1H, $J_{1,2}$ 8.8 Hz, H-1), 6.16 (dd, 1H, $J_{2,3}$ 11.4 Hz, $J_{3,4}$ 3.5 Hz, H-3), 5.83 (d, 1H, $J_{4,5}$ ~3.0 Hz, H-4), 5.04 (dd, 1H, H-2), 4.61 (dd, 1H, $J_{5,6a}$ 6.0 Hz, $J_{6a,6b}$ 10.5 Hz, H-6a), 4.51 (broad t, 1H, H-5), 4.41 (dd, 1H, $J_{5,6b}$ 6.8 Hz, H-6b), 2.21 (s, 3H, OCOC$\underline{H}_3$).
^{13}C NMR (300 MHz, $CDCl_3$): δ_C 94.17 (C-1).
ESMS m/z = 743 $[M+K]^+$, 727 $[M+Na]^+$, 542 $[M-CCl_3CONH_2]^+$ ($C_{32}H_{25}O_{10}N_2Cl_3$ requires m/z = 704).

Benzyl α-D-lyxopyranoside (**13**). Acetyl chloride (3.5 ml) was added to benzyl alcohol (10 ml) cooled at -10 °C and the mixture was allowed to reach room temperature. D-Lyxose (**12**) was added (1g, 6.66 mmol) and the solution left stirring for 5 h. Reduction of the solvent volume by 50% under vacuum and subsequent cooling

overnight in the fridge gave pale yellow crystals that were removed by filtration and recrystallized from ethyl acetate. Spectroscopic data for the white crystals confirmed them to be glycoside **13** (732 mg, 43%) R_f 0.50 (dichloromethane:methanol 10:1), m.p.: 149-151 °C, $[\alpha]_D$ +99.1° (*c* 1, CH_3OH). The filtrate was evaporated and the residue was chromatographed (dichloromethane:methanol step gradient 40:1 to 20:1) to give more compound **13** (307 mg, overall yield 61%). ^1H NMR (360 MHz, CD_3OD): δ_H 7.70 (m, 5H, Ph), 4.75 (d, 1H, $J_{1,2}$ 2.7 Hz, H-1), 4.72 (d, 1H, J_{gem} 11.9 Hz, $PhC\underline{H}_2$), 4.50 (d, 1H, $PhC\underline{H}_2$), 3.82 (dd, 1H, $J_{4,5e}$ 5.0 Hz, $J_{5e,5a}$ 12.0 Hz, H-5e), 3.79 (dd, 1H, $J_{2,3}$ 5.4 Hz, H-2), 3.68 (m, 2H, H-4, H-3), 3.50 (dd, 1H, $J_{4,5a}$ 9.1 Hz, H-5a).

^{13}C NMR (300 MHz, CD_3OD): δ_C 139.02 (Ph quaternary), 129.39, 129.03, 128.77 (Ph methine), 101.11 (C-1), 72.77 (C-3), 71.78 (C-2), 70.19 ($Ph\underline{C}H_2$), 68.55 (C-4), 64.30 (C-5).

Anal. Calcd for $C_{12}H_{16}O_5$ (240.26): C, 59.99; H, 6.71. Found: C, 59.87; H, 6.83.

Benzyl 2,3-O-isopropilidene-α-D-lyxopyranoside (**18**). Benzyl α-D-lyxopyranoside **13** (512 mg, 1.8 mmol) was dissolved in acetone (4 ml) containing 2,2-dimethoxypropane (1.0 ml, 8.13 mmol) and toluenesulfonic acid (40 mg) was added to the solution. After stirring for 2 h, the solution was neutralized with triethylamine (200 µl) and concentrated. The mixture was chromatographed (dichloromethane) to give a white solid (**18**) (540 mg, 95%) as $[\alpha]_D$ +86.8° (*c* 1, $CHCl_3$), R_f 0.50 (dichloromethane:methanol 30:1). ^1H NMR (360 MHz, $CDCl_3$): δ_H 7.35 (m, 5H, Ph), 4.83 (d, 1H, $J_{1,2}$ 2.5 Hz, H-1), 4.80 (d, 1H, J_{gem} 11.9 Hz, $PhC\underline{H}_2$), 4.57 (d, 1H, $PhC\underline{H}_2$), 4.40 (dd, 1H, $J_{3,4}$ 4.4 Hz, $J_{2,3}$ 6.1 Hz, H-3), 4.18(dd, 1H, H-2), 3.85 (dd, 1H, $J_{4,5e}$ 3.4 Hz, $J_{5e,5a}$ 11.1 Hz, H-5e), 3.85-3.75 (m, 1H, H-4), 3.60 (dd, 1H, $J_{4,5a}$ 4.7 Hz, H-5a), 2.90 (d, 1H, $J_{OH,4}$ 7.7 Hz, $O\underline{H}$), 1.44 (s, 3H, $(O_2C(C\underline{H}_3)_2)$), 1.31 (s, 3H, $(O_2C(C\underline{H}_3)_2)$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 136.73 (Ph quaternary), 128.59, 128.26, 128.13 (Ph methine), 109.56 ($O_2\underline{C}(CH_3)_2$), 97.34 (C-1), 76.13 (C-3), 74.45 (C-2), 69.81 ($Ph\underline{C}H_2$), 67.38 (C-4), 63.19 (C-5), 27.44, 25.57 ($O_2C(\underline{C}H_3)_2$).
FABMS (Cleland): m/z = 303 $[M+Na]^+$ and 281 $[M+H]^+$ ($C_{15}H_{20}O_5$ requires m/z = 280).

Benzyl 4-O-benzyl-2,3-O-isopropylidene-α-D-lyxopyranoside (**19**). Acetonide **18** (552 mg, 1.9 mmol) was dissolved in dry DMF (8 ml) and the solution stirred was cooled in an ice bath. Sodium hydride (248 mg) was first added and then benzyl bromide (0.262 ml, 2.2 mmol) was added slowly from a syringe. After 4 h, methanol was added dropwise until no more gas was evolved and then the mixture was poured into ice-water. The mixture was extracted three times (1 x 3 ml) with dichloromethane. The combined extracts were washed with water, dried with anhydrous sodium sulfate and concentrated giving a yellow syrup (**19**) R_f 0.88 (dichloromethane:methanol 30:1). (650 mg, 93%) $[\alpha]_D$ +62.1° (*c* 0.8, $CHCl_3$), ^1H NMR (360 MHz, $CDCl_3$): δ_H 7.38-7.25 (m, 10H, Ph), 4.92 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 4.75 (d, 1H, J_{gem} 12.1 Hz, $PhC\underline{H}_2$), 4.73 (d, 1H, J_{gem} 11.8 Hz, $PhC\underline{H}_2$), 4.69 (d, 1H, $PhC\underline{H}_2$), 4.50 (d, 1H, $PhC\underline{H}_2$), 4.25 (dt, 1H, $J_{3,4}$ 2.0 Hz, H-3), 4.14 (dd, 1H, $J_{2,3}$ 5.7 Hz, H-2), 3.62 (m, 3H, H-4, H-5a, H-5e), 1.40 (s, 3H, ($O_2C(C\underline{H}_3)_2$), 1.35 (s, 3H, ($O_2C(C\underline{H}_3)_2$).
^{13}C NMR (300 MHz, $CDCl_3$): δ_C 138.20, 137.08 (Ph quaternary), 128.51, 128.42, 128.19, 127.95, 127.86, 127.77 (Ph methine), 109.23 ($O_2\underline{C}(CH_3)_2$), 96.97 (C-1), 77.37 (C-3), 75.52 (C-2), 74.67 (C-4), 72.05, 69.22 ($Ph\underline{C}H_2$), 59.20 (C-5), 27.97, 26.39 ($O_2C(\underline{C}H_3)_2$).
Anal. Calcd for $C_{22}H_{26}O_5$ (370.45): C, 71.33; H, 7.07. Found: C, 71.52; H, 7.42

Benzyl 4-O-benzyl-α-D-lyxopyranoside (**20**). Compound **19** (650 mg, 1.8 mmol) was dissolved in aqueous acetic acid (80%, 5 ml) and heated at 60°C overnight. The solvent

was evaporated and the residue chromatographed (pentane:ethyl acetate 2:1) to a white crystalline mass (**20**) (482 mg, 83%), that was recrystallized from ethyl acetate and pentane; m.p.: 100-102 °C, $[\alpha]_D$ +46.5° (*c* 1, $CHCl_3$), R_f 0.47 (pentane:ethyl acetate 1:1). ^1H NMR (360 MHz, $CDCl_3$): δ_H 7.40-7.25 (m, 10H, Ph), 4.72 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.71 (d, 2H, J_{gem} 11.1 Hz, PhC$\underline{H}_2$), 4.63 (d, 1H, J_{gem} 11.8 Hz, PhC$\underline{H}_2$), 4.49 (d, 1H, PhC$\underline{H}_2$), 3.84 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 7.8 Hz, H-3), 3.78 (dd, 1H, H-2), 3.70 (m, 2H, H-4, H-5e), 3.54 (dd, 1H, $J_{4,5a}$ 10.1 Hz, $J_{5a,5e}$ 12.5 Hz, H-5a). ^{13}C NMR (300 MHz, $CDCl_3$): δ_C 138.11, 137.21 (Ph quaternary), 128.63, 128.52, 128.05, 127.96, 127.87 (Ph methine), 98.77 (C-1), 75.35 (C-4), 72.61 (Ph$\underline{C}H_2$), 70.54(C-3), 70.42 (C-2), 69.42 (Ph$\underline{C}H_2$), 60.86 (C-5).

Anal. Calcd for $C_{19}H_{22}O_5$ (330.38): C, 69.07; H, 6.71. Found: C, 69.11; H, 6.92.

Benzyl 2,4-di-O-benzyl-α-D-lyxopyranoside (**21**). A mixture of diol **20** (173 mg, 0.5 mmol), tetrabutylammonium bromide (40 mg, 0.12 mmol), benzyl bromide (0.070 ml, 0.6 mmol) and aqueous sodium hydroxide (15%, 1 ml) in dichloromethane (5 ml) was vigorously stirred at r.t. for 48 h. The organic layer was separated, washed with water, dried and the solvent evaporated. The mixture was chromatographed (pentane:acetone 15:1) to give a white solid (**21**) (96 mg, 44%) $[\alpha]_D$ +53.1°(*c* 0.8, $CHCl_3$), R_f 0.5 7 (pentane:acetone 5:1). Compound **22** was obtained as a by-product as a white solid in 18% yield, as well as a small percentage of the tetra benzylated lyxopyranoside. ^1H NMR (360 MHz, $CDCl_3$): δ_H 7.38-7.25 (m, 15H, Ph), 4.82 (d, 1H, $J_{1,2}$ 3.1 Hz, H-1), 4.75 (d, 1H, J_{gem} 12.0 Hz, PhC$\underline{H}_2$), 4.71 (d, 1H, J_{gem} 11.8 Hz, PhC$\underline{H}_2$), 4.69 (d, 1H, J_{gem} 11.8 Hz, PhC$\underline{H}_2$), 4.63 (d, 1H, PhC$\underline{H}_2$), 4.62 (d, 1H, PhC$\underline{H}_2$), 4.46 (d, 1H, PhC$\underline{H}_2$), 4.10 (dd, 1H, $J_{2,3}$ 3.6 Hz, $J_{3,4}$ 7.7 Hz, H-3), 3.78 (dd, 1H, $J_{4,5e}$ 4.3 Hz, $J_{5e,5a}$ 10.5 Hz, H-5e), 3.76 (dd, 1H, H-2), 3.72 (ddd, 1H, H-4), 3.61 (dd, 1H, H-5a), 2.1 (broad, 1H, O$\underline{H}$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 138.40, 137.89, 137.41 (Ph quaternary), 128.53, 128.49, 127.98, 127.82, 127.76 (Ph methine), 97.41 (C-1), 77.75 (C-3), 75.93 (C-2), 73.43, 72.80 ($PhCH_2$), 70.74 (C-4), 69.37 ($PhCH_2$), 61.21 (C-5).

Anal. Calcd for $C_{26}H_{28}O_5$ (420.51): C, 74.26; H, 6.71. Found: C, 74.36; H, 6.76.

The identification of compound **21** was done by carring out small scale acetylations of the two main products of the reaction (**21** and **22**), to give compounds **23** and **24**.

Benzyl 3-O-acetyl-2,4-di-O-benzyl-α-D-lyxopyranoside (**23**). ^1H NMR (360 MHz, $CDCl_3$): δ_H 7.40-7.20 (m, 15H, Ph), 5.26 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 8.8 Hz, H-3), 4.78 (d, 1H, $J_{1,2}$ 2.9 Hz, H-1), 4.73 (d, 1H, J_{gem} 12.1 Hz, $PhCH_2$), 4.59 (d, 2H, J_{gem} 10.9 Hz, $PhCH_2$), 4.53 (d, 2H, $PhCH_2$), 4.47 (d, 1H, J_{gem} 11.7 Hz, $PhCH_2$), 3.94 (ddd, 1H, $J_{4,5e}$ 5.0 Hz, $J_{4,5a}$ 9.1 Hz, H-4), 3.84 (t, 1H, H-2), 3.79 (dd, 1H, $J_{5e,5a}$ 11.3 Hz, H-5e), 3.66 (dd, 1H, H-5a), 2.20 (s, 3H, $OCOCH_3$).

Benzyl 2-O-acetyl-3,4-di-O-benzyl-α-D-lyxopyranoside (**24**). ^1H NMR (360 MHz, $CDCl_3$): δ_H 7.38-7.20 (m, 15H, Ph), 5.36 (dd, 1H, $J_{1,2}$ 2.33 Hz, $J_{2,3}$ 3.1 Hz, H-2), 4.80 (d, 1H, H-1), 4.78 (d, 1H, J_{gem} 11.2 Hz, $PhCH_2$), 4.70 (d, 2H, J_{gem} 11.9 Hz, $PhCH_2$), 4.69 (d, 2H, J_{gem} 11.4 Hz, $PhCH_2$), 4.61 (d, 2H, $PhCH_2$), 4.55 (d, 2H, $PhCH_2$), 4.45 (d, 2H, $PhCH_2$), 4.01 (m, 1H, H-4), 3.94 (dd, 1H, $J_{3,4}$ 8.8 Hz, H-3), 3.79 (dd, 1H, $J_{4,5e}$ 5.2 Hz, $J_{5e,5a}$ 11.9 Hz, H-5e), 3.61 (dd, 1H, $J_{4,5a}$ 10.2 Hz, H-5a), 2.10 (s, 3H, $OCOCH_3$).

Benzyl 3-O-(3,6-di-O-benzoyl-2-deoxy-4-mesyl-2-phthalimido-β-D-glucopyranosyl)-4-O-benzyl-α-D-lyxopyranoside (**26**) *and Benzyl 2-O-(3,6-di-O-benzoyl-2-deoxy-4-mesyl-2-phthalimido-β-D-glucopyranosyl)-4-O-benzyl-α-D-lyxopyranoside* (**27**). To a solution of the glycosyl imidate **25** (100 mg, 0.13 mmol) and diol **20** (65 mg, 0.18 mmol) in dry dichloromethane (2 ml), was added molecular sieves 4Å (AW, 470 mg) and the mixture was stirred for 2h under a nitrogen atmosphere. The solution was then cooled at -15 °C and TMSOTf (3 µl) was added. After 1 h at -5 °C the mixture was

filtered through Celite and saturated aqueous sodium bicarbonate (1 ml) was added to the filtrate. The organic phase was separated and the aqueous layer was further extracted with dichloromethane (2 x 2 ml). The combined organic extracts were dried with magnesium sulfate, concentrated and chromatographed (pentane:ethyl acetate 3:1). Disaccharides **26** and **27** were each obtained as white foams.

Disaccharide **26** (47 mg, 39%) $[\alpha]_D$ +71.6° (*c* 0.8, $CHCl_3$), R_f 0.67 (pentane:ethyl acetate 3:2). ^1H NMR (500 MHz, $CDCl_3$): δ_H 8.15-7.10 (m, 24H, Ph), 6.30 (dd, 1H, $J_{2',3'}$ 10.5 Hz, $J_{3',4'}$ 9.0 Hz, H-3′), 5.61 (d, 1H, $J_{1',2'}$ 8.4 Hz, H-1′), 5.13 (t, 1H, $J_{3',4'} \approx J_{4',5'}$ 9.5 Hz, H-4′), 4.73 (dd, 1H, $J_{5',6'a}$ 2.1 Hz, $J_{6'a,6'b}$ 12.3 Hz, H-6′a), 4.64 (d, 1H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.57 (dd, 1H, $J_{5',6'b}$ 4.4 Hz, H-6′b), 4.60-4.48 (m, 3H, H-2′, $PhC\underline{H}_2$), 4.35 (d, 1H, $J_{1,2}$ 5.5 Hz, H-1), 4.12-4.06 (m, 3H, H-3, H-5′, $PhC\underline{H}_2$), 3.72-3.65 (m, 3H, H-2, H-4, H-5a), 3.65-3.60 (m, 1H, H-5b), 2.87 (s, 3H, $SO_2C\underline{H}_3$). ^{13}C NMR (300 MHz, $CDCl_3$): δ_C 166.12, 165.51 (C=O), 138.05, 137.09 (Ph quaternary), 134.23, 133.64, 133.25, 133.00, 129.91 (Ph methine), 129.73, 128.65 (Ph quaternary), 128.56, 128.53, 128.39, 128.35, 127.93, 127.88, 127.73, 123.55 (Ph methine), 99.61 (C-1), 98.35 (C-1′), 78.19 (C-3), 74.93 (C-2), 79.08 (C-4), 78.16 ($Ph\underline{C}H_2$), 71.95 (C-4′), 70.35 (C-5′), 70.29 ($Ph\underline{C}H_2$), 68.76 (C-3′), 62.41 (C-5), 62.26 (C-6′), 55.08 (C-2′), 38.92 ($SO_2\underline{C}H_3$).

ESMS m/z = 930 $[M+Na]^+$ ($C_{48}H_{45}O_{15}NS$ requires mz = 907).

Disaccharide **27**: (50 mg, 42%) ^1H NMR (500 MHz, $CDCl_3$): δ_H 8.15-7.13 (m, 24H, Ph), 6.16 (dd, 1H, $J_{2',3'}$ 10.5 Hz, $J_{3',4'}$ 9.3 Hz, H-3′), 5.58 (d, 1H, $J_{1',2'}$ 8.5 Hz, H-1′), 5.11 (t, 1H, $J_{3',4'} \approx J_{4',5'}$ 9.5 Hz, H-4′), 4.88 (dd, 1H, $J_{5',6'a}$ 2.1 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6′a), 4.62 (d, 1H, J_{gem} 12.0 Hz, $PhC\underline{H}_2$), 4.67-4.58 (m, 1H, H-4), 4.56-4.48 (m, 2H, H-6′b, H-2′), 4.53 (d, 1H, J_{gem} 12.0 Hz, $PhC\underline{H}_2$), 4.44 (d, 1H, $J_{1,2}$ 4.1 Hz, H-1), 4.42 (d, 1H, $PhC\underline{H}_2$), 4.20 (d, 1H, $PhC\underline{H}_2$), 4.10 (m, 1H, H-5′), 3.97 (broad s, 1H, H-3), 3.87 (t, 1H, $J_{1,2} \approx J_{2,3}$ 3.8 Hz, H-2), 2.87 (s, 3H, $SO_2C\underline{H}_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 166.23, 166.50 (C=O), 138.49, 137.05 (Ph quaternary), 134.23, 133.70, 133.36 (Ph methine), 131.24 (Ph quaternary), 133.05, 129.98 (Ph methine), 129.61 (Ph quaternary), 128.59, 128.56, 128.44, 128.32, 127.80, 127.72, 127.61, 127.50, 123.63 (Ph methine), 98.30 (C-1), 97.07 (C-1′), 79.99 (C-3), 75.88 (C-2), 74.69 (C-4), 72.82 ($PhCH_2$), 72.47 (C-4′), 70.41 (C-5′), 69.81 (C-3′), 69.18 ($PhCH_2$), 62.04 (C-5), 61.70 (C-6′), 54.80 (C-2′), 38.96 (SO_2CH_3).

ES m/z = 930 $[M+Na]^+$ ($C_{48}H_{45}O_{15}NS$ requires mz = 907).

Benzyl 3-O-(3,6-di-O-benzoyl-2-deoxy-4-mesyl-2-phthalimido-β-D-glucopyranosyl)-2,4-di-O-benzyl-α-D-lyxopyranoside (**28**). Molecular sieves 4Å (AW, 4.2 g) was added to a solution of glycosyl imidate **25** (881 mg, 1.2 mmol) and the selectively protected alcohol **21** (610 mg, 1.4 mmol) in dry dichloromethane (20 ml), and the mixture was stirred for 2h under a nitrogen atmosphere. The solution was cooled to -15 °C, TMSOTf (30 µl) was added and after 1 h at -5 °C the solution was filtered through Celite and saturated aqueous sodium bicarbonate (10 ml) was added to the filtrate. The organic layer was separated and the aqueous layer was further extracted with dichloromethane (2 x 15 ml). The combined organic extracts were dried with magnesium sulfate, concentrated and chromatographed (pentane:ethyl acetate 3:1). Disaccharide **28** (1.04 g, 88%) was obtained as a white foam $[\alpha]_D$ +71.6° (*c* 0.8, $CHCl_3$), R_f 0.76 (pentane:ethyl acetate 3:2). ^1H NMR (500 MHz, $CDCl_3$): δ_H 8.12-7.04 (m, 29H, Ph), 6.36 (broad t, 1H, $J_{2',3'} \approx J_{3',4'}$ 9.6 Hz, H-3′), 5.52 (broad d, 1H, H-1′), 5.10 (t, 1H, $J_{3',4'} \approx J_{4',5'}$ 9.5 Hz, H-4′), 4.72 (dd, 1H, $J_{5',6'a}$ 2.1 Hz, $J_{6'a,6'b}$ 12.2 Hz, H-6′a), 4.64 (d, 1H, J_{gem} 11.8 Hz, $PhCH_2$), 4.54 (dd, 1H, $J_{5',6'b}$ 4.6 Hz, H-6′b), 4.50 (dd, 1H, $J_{1',2'}$ 8.4 Hz, $J_{2',3'}$ 10.7 Hz, H-2′), 4.42 (broad d, 1H, $PhCH_2$), 4.34 (broad s, 1H, H-1), 4.40-4.30 (broad m, 1H, $PhCH_2$), 4.21 (d, 1H, J_{gem} 12.2 Hz, $PhCH_2$), 4.08 (d, 2H, J_{gem} 12.3 Hz, $PhCH_2$), 4.06 (ddd, 1H, H-5′), 3.83 (broad s, 1H, H-3), 3.72-

3.62 (m, 2H, H-5a, H-5e), 3.56 (broad s, 1H, H-4), 3.44 (broad dd, 1H, $J_{1,2}$ 2.8 Hz, $J_{2,3}$ 6.4 Hz, H-2), 2.82 (s, 3H, $SO_2C\underline{H}_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 167.76, 166.10, 165.48 (C=O), 138.23, 138.13, 137.70 (Ph quaternary), 133.97, 133.60, 133.24, 129.97, 129.89 (Ph methine), 129.79, 128.73, (Ph quaternary), 128.56, 128.52, 128.27, 128.22, 127.94, 127.69, 127.64, 127.55, 127.52, 123.37 (Ph methine), 99.86 (C-1), 99.13 (C-1′), 78.62 (C-3), 75.17 (C-2), 75.00 (C-4, C-4′), 72.96 ($Ph\underline{C}H_2$), 71.85 (C-5′, $Ph\underline{C}H_2$), 70.72 ($Ph\underline{C}H_2$), 70.23 (C-3′), 62.92 (C-5), 62.36 (C-6′), 55.10 (C-2′), 38.93 ($SO_2\underline{C}H_3$). *Anal.* Calcd for $C_{55}H_{51}O_{15}N\,S$ (998.07): C, 66.19; H, 5.15; N, 1.40; S, 3.21. Found: C, 65.85; H, 5.23; N, 1.45; S, 2.92.

Benzyl 3-O-(4-O-acetyl-3,6-di-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2,4-di-O-benzyl-α-D-lyxopyranoside (**29**). Method A: To a solution of the disaccharide mesylate **28** (356 mg, 0.3 mmol) in dry DMF (3 ml), were added dicyclohexano-18-crown-6 (20 mg, 0.05 mmol) together with potassium acetate (122 mg, 1.2 mmol). The mixture was kept at 130 °C for 24 h, cooled, poured into ice-water and extracted with ethyl acetate; the extracts were combined, washed with water, dried and concentrated. The residue was chromatographed (pentane:ethyl acetate 3:1) to give compound **29** as a white foam (203 mg, 60%) $[\alpha]_D$ +23.1° (*c* 0.8, $CHCl_3$), R_f 0.3 (pentane:ethyl acetate 3:1).

Method B: to a solution of the galactosyl imidate **32** (2.2 g, 3.12 mmol) and alcohol **21** (1.44 g, 3.43 mmol) in dry dichloromethane (44 ml), was added molecular sieves 4Å (AW, 10.5 g) and the mixture was stirred for 2h under a nitrogen atmosphere. The mixture was cooled at -15 °C and TMSOTf (45 µl) was added to it. After 1 h at -5 °C the solution was filtered through Celite and worked up in the manner described for diaccharide **29**. After concentration, the residue was chromatographed (4:1 hexane:acetone) to give disaccharide **29** (2.6 g, 87%). ^1H NMR (500 MHz, $CDCl_3$): δ_H

8.12-7.04 (m, 29H, Ph), 6.24 (broad d, 1H, H-3′), 5.77 (d, 1H, $J_{3',4'} \approx J_{4',5'}$ 3.7 Hz, H-4′), 5.49 (broad s, 1H, H-1′), 4.74 (dd, 1H, $J_{1',2'}$ 8.5 Hz, $J_{2',3'}$ 11.3 Hz, H-2′), 4.67 (d, 1H, J_{gem} 11.7 Hz, $PhC\underline{H}_2$), 4.51(dd, 1H, $J_{5',6'a}$ 5.2 Hz, $J_{6'a,6'b}$ 9.9 Hz, H-6′a), 4.40 (broad s, 2H, H-1, $PhC\underline{H}_2$), 4.32-4.22 (m, 2H, H-5′, H-6′b), 4.26 (d, 1H, J_{gem} 12.0 Hz, $PhC\underline{H}_2$), 4.24 (d, 1H, J_{gem} 11.4 Hz, $PhC\underline{H}_2$), 4.10 (d, 2H, J_{gem} 12.3 Hz, $PhC\underline{H}_2$) 3.86 (broad s, 1H, H-3), 3.78-3.68 (m, 2H, H-5a, H-5e), 3.62 (broad s, 1H, H-4), 3.44 (dd, 1H, $J_{1,2}$ 2.7 Hz, $J_{2,3}$ 6.1 Hz, H-2), 2.16 (s, 3H, $OCOC\underline{H}_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 169.97, 168.42, 168.01, 166.02, 165.04 (C=O), 138.26, 138.08, 137.77 (Ph quaternary), 133.97, 133.81, 133.35 (Ph methine), 131.93, 131.49 (Ph quaternary), 129.76, 129.67 (Ph methine), 129.49, 129.06 (Ph quaternary), 128.55, 128.47, 128.30, 128.23, 128.08, 127.73, 127.70, 127.54, 127.49, 123.29, 123.17 (Ph methine), 100.05 (C-1), 99.70 (C-1′), 78.39 (C-3), 75.13 (C-2, C-4), 73.01, 71.66 ($Ph\underline{C}H_2$), 70.99 (C-5′), 70.78 (PhCH2), 68.31 (C-3′), 66.89 (C-4′), 63.08 (C-5), 61.89 (C-6′), 51.78 (C-2′), 20.72 ($OCO\underline{C}H_3$).

Anal. Calcd for $C_{56}H_{51}O_{14}N$ (962.02): C, 69.92; H, 5.34; N, 1.46. Found: C, 69.97; H, 5.29; N, 1.46.

Benzyl 3-O-(2-amino-2-deoxy-β-D-galactopyranosyl)-2,4-di-O-benzyl-α-D-lyxopyranoside (**33**). The protected disaccharide **29** (500 mg, 0.5 mmol) was suspended in ethanol (10 ml). Hydrazine hydrate (1 ml) was added and the mixture was refluxed at 78 °C for 4 h. The solution was evaporated and the residue was chromatographed using Iatrobeads (ethyl acetate to ethyl acetate, methanol, triethylamine; step gradient 100 to 70:10:0.3) to give compound **33** (232 mg, 80%) as a white foam. $[\alpha]_D$ +5.5° (*c* 1, CH_3OH), R_f 0.37 (ethyl acetate:methanol:triethylamine 70:25:0.3). ^1H NMR (600 MHz, CD_3OD): δ_H 7.38-7.25 (m, 15H, Ph), 4.86 (d, 1H, $J_{1,2}$ 5.8 Hz, H-1), 4.80 (broad d, 2H, $PhC\underline{H}_2$), 4.72 (d, 1H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.64 (d, 1H, J_{gem} 11.8 Hz, $PhC\underline{H}_2$), 4.60 (d, 1H, J_{gem} 11.2 Hz, $PhC\underline{H}_2$), 4.56 (d, 1H,

PhC$\underline{H}_2$), 4.32 (d, 1H, $J_{1',2'}$ 8.1 Hz, H-1′), 4.15 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 5.9 Hz, H-3), 3.86 (m, 1H, $J_{4,5e}$ 2.2 Hz, $J_{4,5a}$ 3.1 Hz, H-4), 3.82 (dd, 1H, $J_{5a,5e}$ 12.0 Hz, H-5e), 3.80-3.65 (m, 5H, H-2, H-5a, H-4′, H-6′a, H-6′b), 3.46 (dt, 1H, $J_{5',4'}$ 0.9 Hz, $J_{5',6'a} \approx J_{5',6'b}$ 6.6 Hz, H-5′), 3.37 (dd, 1H, $J_{2',3'}$ 10.2 Hz, $J_{3',4'}$ 3.3 Hz, H-3′), 3.12 (dd, 1H, $N\underline{H}_2$).

^{13}C NMR (300 MHz, CD_3OD): δ_C 139.74, 139.69, 139.05 (Ph quaternary), 129.40, 129.29, 129.11, 129.01, 128.79 (Ph methine), 105.33 (C-1), 100.22 (C-1′), 78.59 (C-3), 77.22 (C-2), 77.05 (C-5′), 74.62 (C-3′), 74.11, 73.35, 71.28 (Ph$\underline{C}H_2$), 69.39 (C-4′), 63.65 (C-5), 62.52 (C-6′), 54.92 (C-2′).

ESMS: m/z = 582 $[M+H]^+$, 474 $[M-PhCH_2O]^+$ ($C_{32}H_{39}O_9N$ requires m/z = 581).

Benzyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-2,4-di-O-benzyl-α-D-lyxopyranoside (**34**). Crude disaccharide **33** was acetylated overnight in a solution of pyridine (3 ml) and acetic anhydride (1 ml). After co-evaporation with toluene, the residue was chromatographed (pentane:ethyl acetate 3:1) giving tri-*O*-acetyl disaccharide **34** (315 mg, 81% from **29**). $[\alpha]_D$ +5.7° (*c* 1, $CHCl_3$), R_f 0.76 (dichloromethane:methanol 10:1) as a transparent residue. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.61-7.20 (m, 15H, Ph), 5.26 (m, 2H, $N\underline{H}COCH_3$, H-4′), 4.90 (d, 1H, J_{gem} 11.9 Hz, PhC$\underline{H}_2$), 4.83-4.77 (m, 2H, J_{gem} 10.8 Hz, PhC$\underline{H}_2$, H-3′), 4.65 (d, 1H, J_{gem} 12.1 Hz, PhC$\underline{H}_2$), 4.65 (d, $J_{1,2}$ 7.0 Hz, H-1), 4.60 (d, 1H, $J_{1',2'}$ 8.7 Hz, H-1′), 4.59 (d, 1H, J_{gem} 10.8 Hz, PhC$\underline{H}_2$), 4.54 (d, 1H, J_{gem} 12.1 Hz, PhC$\underline{H}_2$), 4.00 (d, 1H, J_{gem} 11.9 Hz, PhC$\underline{H}_2$), 4.11 (dd, 1H, $J_{2',3'}$ 10.9 Hz, H-2′), 4.10-4.03 (m, 3H, H6′a, H6′b, H-3), 3.84 (broad dd, 1H, $J_{4,5a}$ 2.8 Hz, $J_{5a,5e}$ 12.3 Hz, H-5a), 3.78 (t, 1H, $J_{5',6'a} \approx J_{5',6'b}$ 7.0Hz, H-5′), 3.74 (dd, 1H, $J_{4,5e}$ 2.1 Hz, H-5e), 3.71 (dd, 1H, $J_{1,2}$ 7.0 Hz, $J_{2,3}$ 3.0 Hz, H-2), 3.65 (broad s, 1H, H-4), 2.40, 2.12, 2.10, 2.00, 1.92 (s, 3H, $NHCOC\underline{H}_3$, 12 H, $OCOC\underline{H}_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 170.51, 170.40, 170.37, 170.29 (C=O), 138.17, 138.07, 137.77 (Ph quaternary), 128.76, 128.49, 128.45, 128.35, 128.27, 127.92, 127.82, 127.78 (Ph methine), 102.80 (C-1′), 99.99 (C-1), 77.58 (C-3), 76.79 (C-2), 75.45 (C-4), 74.16, 71.59 ($PhCH_2$), 71.16 (C-5′), 70.98 (C-3′), 70.57 (PhCH2), 66.59 (C-4′), 62.83 (C-5), 61.37 (C-6′), 50.69 (C-2′), 22.98 ($NHCOCH_3$), 20.76, 20.71, 20.69 ($OCOCH_3$).

FABMS (Cleland): m/z = 788 $[M+K]^+$, 772 $[M+Na]^+$ and 642 $[M-PhCH_2OH]^+$ ($C_{40}H_{47}O_{13}N$ requires m/z = 749).

3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-D-lyxopyranose (**35**). Disaccharide **34** (88 mg, 0.12 mmol) was dissolved in glacial acetic acid (15 ml) and hydrogenated over Pd/C (180 mg) for 4 h. The solution was filtered through Celite and co-evaporated with toluene and chromatographed on Iatrobeads (dichloromethane:methanol 10:1) to give partially acetylated disaccharide **35** (38 mg, 68%) as a foam R_f 0.38 (dichloromethane:methanol 20:1). ^{1}H NMR (500 MHz, CD_3OD): δ_H 5.34 (d, 1H, $J_{3',4'}$ 3.4 Hz, H-4′), 5.08 (dd, 1H, $J_{2',3'}$ 11.3 Hz, H-3′), 4.80 (d, 1H, $J_{1,2}$ 5.8 Hz, H-1), 4.78 (d, 1H, $J_{1',2'}$ 8.5 Hz, H-1′), 4.14 (m, 2H, H-6′a, H-6′b), 4.11 (dd, 1H, H-2′), 4.04 (t, 1H, $J_{5',6'a} \approx J_{5',6'b}$ 6.5 Hz, H-5′), 3.91 (dd, 1H, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 6.0 Hz, H-3), 3.85 (ddd, 1H, $J_{4,5e}$ 3.3 Hz, $J_{4,5a}$ 5.4 Hz, H-4), 3.77 (dd, 1H, $J_{5e,5a}$ 11.8 Hz, H-5e), 3.72 (dd, 1H, H-2), 3.65 (dd, 1H, H-5a), 2.13, 2.02, 1.95 (s, 12H, $OCOCH_3$).

^{13}C NMR (300 MHz, CD_3OD): δ_C 174.25, 172.16, 172.06, 171.73 (C=O), 103.05 (C-1′), 95.99 (C-1), 81.66 (C-3), 72.01 (C-5′), 71.98 (C-3′), 68.65 (C-4), 68.01 (C-4′), 65.16 (C-5), 62.81 (C-6′), 51.96 (C-2′), 23.03 ($NHCOCH_3$), 20.55, 20.52, 20.39 ($OCOCH_3$).

FABMS (Cleland): m/z = 502 $[M+Na]^+$ and 480 $[M+H]^+$ ($C_{19}H_{29}O_{13}N$ requires m/z = 479).

Benzyl 3-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-2,4-di-O-benzyl-α-D-lyxopyranoside (**36**). Disaccharide **34** (1.1 g, 1.46 mmol) was dissolved in methanol (12 ml) and a solution of sodium methoxide was added until the pH was ca. 8.5. When the deacetylation was complete the solution was neutralized with methanol-washed Rexyn 101 (H^+), filtered and the combined filtrates concentrated giving the partially deprotected disaccharide **36** (870 mg, 95%) as a transparent residue. $[\alpha]_D$ +20.8° (*c* 1, CH_3OH), R_f 0.61 (dichloromethane:methanol 10:1). ^1H NMR (500 MHz, CD_3OD): δ_H 7.40-7.23 (m, 15H, Ph), 5.48 (s, 1H, $NHCOCH_3$), 4.80 (broad d, 1H, $PhCH_2$), 4.77 (d, $J_{1,2}$ 5.8 Hz, H-1), 4.71 (d, 2H, J_{gem} 12.3 Hz, $PhCH_2$), 4.62 (d, 1H, $PhCH_2$), 4.59 (d, 1H, $J_{1',2'}$ 8.2 Hz, H-1′), 4.53 (d, 1H, J_{gem} 11.9 Hz, $PhCH_2$), 4.49 (d, 1H, $PhCH_2$), 4.18 (dd, 1H, $J_{2,3}$ 5.5 Hz, $J_{3,4}$ 2.8 Hz, H-3), 3.91 (broad t, 1H, H-2′), 3.85 (dd, 1H, $J_{4,5e}$ 3.4 Hz, $J_{5e,5a}$ 7.8 Hz, H-5e), 3.82 (d, 1H, $J_{3',4'}$ 3.3 Hz, H-4′), 3.81-3.77 (m, 1H, H-4), 3.77-3.67 (m, 4H, H-2, H-5a, H-6′a, H-6′b), 3.56 (dd, 1H, $J_{2',3'}$ 10.5 Hz, H-3′), 3.48 (ddd, 1H, $J_{5',6'a}$ 7.0 Hz, $J_{5',6'b}$ 9.0 Hz, H-5′), 1.70 (s, 3H, $NHCOCH_3$). ^{13}C NMR (300 MHz, $CDCl_3$): δ_C 174.38 (C=O), 137.82, 137.58, 137.17 (Ph quaternary), 128.78, 128.56, 128.52, 128.08, 128.02, 127.97, 127.91 (Ph methine), 103.08 (C-1′), 100.06 (C-1), 78.98 (C-3), 77.18 (C-2), 75.13 (C-3′), 74.96 ($PhCH_2$), 74.88 (C-5′), 74.79 (C-4), 71.12, 70.75 (PhCH2), 67.90 (C-4′), 62.87 (C-5), 62.52 (C-6′), 50.69 (C-2′), 21.96 ($NHCOCH_3$).
FABMS (Cleland): m/z = 662 $[M+K]^+$, 646 $[M+Na]^+$ and 624 $[M+H]^+$ ($C_{34}H_{41}O_{10}N$ requires m/z = 623).

3-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D-lyxopyranose (**37**). Compound **36** (121 mg, 0.19 mmol) was dissolved in acetic acid (15 ml); 247 mg of Pd/C were added and the solution hydrogenated for 6h. The mixture was purified in Iatrobeads (dichloromethane:methanol 2:1) to give the fully deprotected disaccharide **37** (50 mg,

72%) as a white powder R_f 0.45 (chloroform:methanol:water 6:6:0.5). ^{1}H NMR (500 MHz, D_2O): δ_H 4.92 (d, 1H, $J_{1,2}$ 5.2 Hz, H-1, a), 4.84 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1, b), 4.63 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1′), 4.62 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1′), 2.05 (s, 3H, $NHCOCH_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 175.99 175.88 (C=O), 102.42 (C-1′), 101.01 (C-1′), 94.90 (2C, C-1α, C-1β), 23.15 ($NHCOCH_3$).

FABMS (Cleland): m/z = 391 $[M+K]^+$, 376 $[M+Na]^+$ and 354 $[M+H]^+$ ($C_{13}H_{23}O_{10}N$ requires m/z = 353).

4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-1,2,3,6-tetra-O-acetyl-β-D-(1-^{13}C)galactopyranose (**40**). The hexosaminyl-lyxose **37** (250 mg, 0.7 mmol) was added slowly as a solid into a round bottom flask containing a solution of ^{13}C potassium cyanide at pH 7.2-7.5, prepared by dissolving the cyanide salt (281 mg, 4.3 mmol) in the minimum amount of water and lowering the pH with 5M acetic acid. Each addition of compound was followed by pH adjustment with 5M acetic acid or 1M sodium hydroxide as required. After 20 minutes the pH was adjusted to 4.8 and nitrogen was bubbled through the solution to eliminate excess cyanide. The purged air flow was subsequently bubbled through a series of three traps containing methanolic potassium hydroxide. After 3 h the volume of the solution had been reduced to half its original volume and added by syringe to a flask containing previously hydrogenated (10 min) Pb/barium sulfate (44 mg) in 5 ml of water. The mixture was then hydrogenated for 4 h and treated with resins following the literature procedures reported to give compounds **38** and **39**. Acetic anhydride (2.3 ml) was added to the anomeric mixture of disaccharides **38** and **39** dissolved in pyridine (5 ml) and the reaction mixture was left overnight at r.t. After co-evaporation of the solution with toluene, the residue was chromatographed (ethyl acetate:toluene 3:1) to give compounds **40** and **41** as clear residues.

Compound **40**: (95 mg, 35% overall yield), $[\alpha]_D$ +8.2° (*c* 1, $CHCl_3$), R_f 0.62 (acetone:toluene 2:1). ^{1}HNMR (500 MHz, $CDCl_3$): δ_H 5.84 (dd, 1H, $J_{2',3'}$ 11.3 Hz, $J_{3',4'}$ 3.4 Hz, H-3′), 5.66 (dd, 1H, $J_{1,2}$ 8.4 Hz, $^1J_{H,^{13}C}$ 166.7 Hz, H-1), 5.64 (d, 1H, $J_{NH,2'}$ 7.1 Hz, N<u>H</u>COCH$_3$), 5.37 (ddd, 1H, $^2J_{H,^{13}C}$ 6.4 Hz, $J_{2,3}$ 10.7 Hz, H-2), 5.36 (d, 1H, H-4′), 5.09 (d, 1H, $J_{1',2'}$ 8.1 Hz, H-1′), 4.98 (ddd, 1H, $^3J_{H,^{13}C}$ 1.1 H z, $J_{3,4}$ 2.9 Hz, H-3), 4.30 (dd, 1H, $J_{5,6a}$ 4.8 Hz, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.20 (dd, 1H, $J_{5,6b}$ 7.0Hz, H-6b), 4.16 (d, 1H, H-4), 4.04 (d, 2H, $J_{5',6'a} \approx J_{5',6'b}$ 7.0 Hz, H-6′a, H-6′b), 3.89 (t, 1H, H-5′), 3.87 (dt, 1H, $^3J_{H,^{13}C}$ 1.5 Hz, H-5), 3.39 (ddd, 1H, H-2′), 2.12, 2.09, 2.04, 2.02, 2.01, 1.97, 1.96 (s, 24H, OCOC<u>H</u>$_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 171.15, 170.83, 170.65, 170.59, 170.48, 170.13, 169.81, 169.49 (C=O), 99.13 (C-1′), 92.00 (^{13}C-1), 73.50 (C-4), 73.28 (C-3), 73.08 (C-5), 72.84 (C-2), 70.56 (C-5′), 68.16 (C-3′), 66.96 (C-4′), 63.02 (C-6), 61.51 (C-6′), 53.63 (C-2′), 23.46 (NHCO<u>C</u>H$_3$), 20.95, 20.85, 20.77, 20.69, 20.66, 20.55 (7, OCO<u>C</u>H$_3$).

ESMS (high res) m/z = 701.210190 $[M+Na]^+$ (Calcd 701.209839 for $C_{27}{}^{13}CH_{39}NO_{18}Na$).

Compound **41** *(Acetyl 4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-α/β-D-(1-^{13}C)talopyranoside)*: ^{1}HNMR (500 MHz, $CDCl_3$): 6.09 (dd, 1H, $J_{1,2}$ 3.6 Hz, $^1J_{1_H,^{13}C}$ 176.7 Hz, H-1, α), 5.95 (broad d, 1H, $^1J_{1_H,^{13}C}$ 169.3 Hz, H-1, β). ^{13}C NMR (300 MHz, $CDCl_3$): 90.01 (^{13}C-1 β), 89.30 (^{13}C-1 α).

4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-α-D-(1-^{13}C)galactopyranosyl trichloroacetimidate (**43**). Compound **40** (85 mg, 0.125 mmol)was dissolved in a solution of hydrazine acetate (10.8 mg) in DMF (2 ml) and stirred for 1.5 h at r.t. The solution was then concentrated, diluted with ethyl acetate (3 ml), washed with a saturated solution of sodium chloride (2 x 1 ml), dried with sodium

sulfate and evaporated, to give a pale yellow residue R_f 0.34, (dichloromethane:acetone 2:1). Crude **42** dried overnight under vacuum was dissolved in dichloromethane (2 ml), and cooled at 0 °C under nitrogen. Trichloroacetonitrile (0.245 ml) followed by DBU (1.5 µl) were added to the solution of **42** and the mixture was left to stand for 45 minutes at 0 °C. Concentration and chromatography (dichloromethane:acetone 3:1) of the residue gave the expected product **43** (72 mg, 73%) as a clear residue R_f 0.55 (dichloromethane:acetone 2:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ_H 8.62 (s, 1H, C=N<u>H</u>), 6.48 (dd, 1H, $J_{1,2}$ 3.7 Hz, $^1J_{^1H,^{13}C}$ 179.5 H z, H-1), 5.80 (dd, 1H, $J_{2',3'}$ 11.3 Hz, $J_{3',4'}$ 3.5 Hz, H-3′), 5.65 (d, 1H, $J_{NH,2'}$ 7.3 Hz, N<u>H</u>COCH$_3$), 5.53 (dd, 1H, $J_{2,3}$ 10.8 Hz, H-2), 5.37 (d, 1H, H-4′), 5.33 (dd, 1H, $J_{3,4}$ 2.5 Hz, H-3), 5.07 (d, 1H, $J_{1',2'}$ 8.2 Hz, H-1′), 4.32-4.26 (m, 2H, H-4, H-6a), 4.18 (m, H-5, H-6b), 4.04 (d, 2H, $J_{5',6'a} = J_{5',6'b}$ 6.8 Hz, H-6′a, H-6′b), 3.90 (dt, 1H, H-5′), 3.40 (ddd, 1H, H-2′), 2.14, 2.12, 2.02, 2.00, 1.99, 1.98, 1.94 (s, 21H, OCOC<u>H</u>$_3$).

^{13}C NMR ($CDCl_3$): δ_C 99.25 (C-1′), 93.94 (^{13}C-1).

ESMS (high res) m/z = 802.109650 $[M+Na]^+$ (Calcd 802.108906 for $C_{27}{}^{13}CH_{37}N_2O_{17}Na\ Cl_{27}$).

Allyl 4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-(1-^{13}C)galactopyranoside (**44**). Molecular sieves 4Å (AW, 340 mg) was added to a solution of imidate **43** (64 mg, 0.08 mmol) and allyl alcohol (0.5 ml) in dry dichloromethane (3 ml), and the mixture was stirred for 2h under a nitrogen atmosphere. The mixture was then cooled at -15 °C and TMSOTf (1.5 µl) was added to it. After 1 h at -10 °C the mixture was filtered through Celite and the filtrate was treated with saturated aqueous sodium bicarbonate (1 ml). The organic layer was separated and the aqueous layer was further extracted with dichloromethane (2 x 2 ml). The combined organic layers were dried with magnesium sulfate, concentrated and chromatographed (ethyl acetate:toluene 3:1). Allyl glycoside **44** (40 mg, 72%) was obtained as a clear

residue $[\alpha]_D$ -8.5° (*c* 0.5, $CHCl_3$), R_f 0.56 (toluene:acetone 1:1). ^1H NMR (600 MHz, $CDCl_3$): δ_H 5.92 (dd, 1H, $J_{3',4'}$ 3.4 Hz, $J_{2',3'}$ 11.3 Hz, H-3′), 5.84 (dddd, 1H, $J_{c,d}$ 4.9 Hz, $J_{c,e}$ 6.1 Hz, $J_{b,c}$ 10.4 Hz, $J_{a,c}$ 17.3 Hz, H-c), 5.66 (d, 1H, $J_{NH,2'}$ 6.9 Hz, N<u>H</u>COCH$_3$), 5.37 (d, 1H, H-4′), 5.28 (ddd, 1H, $^2J_{H,^{13}C}$ 6.4 Hz, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 10.5 Hz, H-2), 5.25 (ddd, 1H, $J_{a,d} \approx J_{a,e}$ 1.7 Hz, $J_{a,b}$ 3.2 Hz, H-a), 5.18 (ddd, 1H, $J_{b,d} \approx J_{b,e}$ 1.4 Hz, H-b), 5.13 (d, 1H, $J_{1',2'}$ 8.2 Hz, H-1′), 4.93 (ddd, 1H, $^3J_{H,^{13}C}$ 1.1 Hz, $J_{3,4}$ 2.8 Hz, H-3), 4.48 (dd, 1H, $^1J_{H,^{13}C}$ 160.3 Hz, H-1), 4.36 (m, 1H, H-d), 4.31-4.23 (m, 2H, H-6a, H-6b), 4.13 (m, 1H, H-e), 4.12 (d, 1H, H-4), 4.03 (d, 2H, $J_{5',6'a} \approx J_{5',6'b}$ 6.6 Hz, H-6′a, H-6′b), 3.89 (t, 1H, H-5′), 3.71 (dt, 1H, $^3J_{H,^{13}C}$ 1.4 Hz, $J_{5,6a} \approx J_{5,6b}$ 6.0 Hz, H-5), 3.33 (ddd, 1H, H-2′), 2.12, 2.09, 2.06, 2.03, 2.02, 1.98, 1.97 (s, 21H, OCOC<u>H</u>$_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 171.32, 170.92, 170.55, 170.47, 170.15, 169.74, 169.56 (C=O), 133.55 (OCH$_2$<u>C</u>H=CH$_2$), 117.52 (OCH$_2$CH=<u>C</u>H$_2$), 99.90 (^{13}C-1), 98.92 (C-1′), 73.26 (C-4), 72.92 (C-3), 72.20 (C-5), 70.47 (C-5′), 70.06 (O<u>C</u>H$_2$CH=CH$_2$), 69.52 (C-2), 68.06 (C-3′), 67.04 (C-4′), 63.20 (C-6), 61.51 (C-6′), 53.74 (C-2′), 23.51 (NHCO<u>C</u>H$_3$), 20.66 to 20.20 (6C, OCO<u>C</u>H$_3$).

ESMS (high res) m/z = 699.230810 $[M+Na]^+$ (Calcd 699.230574 for $C_{28}{}^{13}CH_{41}NO_{17}Na$).

Allyl 4-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-(1-^{13}C)galactopyranoside (**45**). Disaccharide **44** (40 mg, 0.06 mmol) was dissolved in methanol (2 ml) and a solution of sodium methoxide was added until the pH was ca. 8.5. When the deacetylation was complete the solution was neutralized with methanol-washed Rexyn 101 (H^+), filtered and the filtrates and washings were concentrated to give compound **45** (24 mg, 96%) as a transparent residue. $[\alpha]_D$ -4.5° (*c* 0.5, CH_3OH), R_f 0.48 (chloroform:methanol 1:1). ^1H NMR (600 MHz, CD_3OD): δ_H 5.94 (ddd, 1H, $J_{c,d} \approx J_{c,e}$ 5.6 Hz, $J_{b,c}$ 10.6 Hz, $J_{a,c}$ 17.0 Hz, H-c), 5.31 (ddd, 1H, $J_{a,d} \approx J_{a,e}$ 1.8 Hz, $J_{a,b}$ 3.4 Hz,

H-a), 5.14 (ddd, 1H, $J_{b,d} \approx J_{b,e}$ 1.5 Hz, H-b), 4.64 (d, 1H, $J_{1',2'}$ 8.3 Hz, H-1′), 4.31 (dddd, $J_{d,e}$ 13.0 Hz, H-d), 4.24 (dd, 1H, $^1J_{^1H,^{13}C}$ 158.6 Hz, $J_{1,2}$ 7.8 Hz, H-1), 4.13 (dddd, 1H, H-e), 4.02 (d, 1H, $J_{3,4}$ 3.5 Hz, H-4), 3.87 (dd, 1H, $J_{5',6'a}$ 7.3 Hz, $J_{6'a,6'b}$ 11.3 Hz, H-6′a), 3.86 (m, 1H, H-2′), 3.80 (dd, 1H, $J_{5,6a}$ 7.9 Hz, $J_{6a,6b}$ 11.2 Hz, H-6a), 3.76 (d, 1H, $J_{3',4'}$ 3.3 Hz, H-4′), 3.70 (dd, 1H, $J_{5,6b}$ 4.4 Hz, H-6b), 3.62 (dd, 1H, $J_{5',6'b}$ 5.1 Hz, H-6′b), 3.61 (m, 1H, H-3), 3.59 (dd, 1H, $J_{2',3'}$ 10.5 Hz, H-3′), 3.52 (m, 1H, H-5), 3.50 (m, 1H, H-5′), 3.46 (m, 1H, H-2), 2.02 (s, 3H, $NHCOC\underline{H}_3$)

^{13}C NMR (300 MHz, CD_3OD): δ_C 171.32 (C=O), 135.55 ($OCH_2\underline{C}H=CH_2$), 117.52 ($OCH_2CH=\underline{C}H_2$), 104 (C-1′), 103 (^{13}C-1), 78 (C-4), 76 (C-5), 75 (C-5′), 74 (C-3), 74 (C-3′), 72 (C-2), 70 ($O\underline{C}H_2CH=CH_2$), 69 (C-4′), 62 (C-6), 61. (C-6′), 55 (C-2′), 23.51 ($NHCO\underline{C}H_3$).

ESMS (high res) m/z = 425.184910 $[M+H]^+$ (Calcd 425.185241 for $C_{16}{}^{13}CH_{30}NO_{11}$).

Benzyl 3-O-(2-(1,2 -^{13}C)acetamido-2-deoxy–β-D-galactopyranosyl)-2,4-di-O-benzyl-α-D-lyxopyranoside (**46**). Compound **33** (650 mg, 1.12 mmol) was dissolved in dry dichloromethane (2.5 ml) containing triethylamine (0.5 ml). The mixture was cooled to -20 °C and from a syringe a solution of acetyl chloride (120 µl, 1.5 mmol) in dry dichloromethane (1 ml) was added dropwise. After 1 h at -15 °C the solution was worked up by extraction with 2M hydrochloric acid (2 x 1 ml), saturated sodium bicarbonate (2 x 1 ml), sodium chloride solution (2 x 1 ml) and the sodium sulphate dried solution was evaporated to give **46** as a clear residue (660 mg, 95%). ^1H NMR (500 MHz, CD_3OD): δ_H 7.40-7.25 (m, 15H, Ph), 4.80 (broad d, 1H, $PhC\underline{H}_2$), 4.77 (d, $J_{1,2}$ 5.8 Hz, H-1), 4.70 (d, 2H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.68 (d, 1H, $PhC\underline{H}_2$), 4.64 (d, 1H, J_{gem} 11.6 Hz, $PhC\underline{H}_2$), 4.62 (d, 1H, $PhC\underline{H}_2$), 4.60 (d, 1H, $J_{1',2'}$ 8.4 Hz, H-1′), 4.52 (d, 1H, J_{gem} 11.8 Hz, $PhC\underline{H}_2$), 4.19 (dd, 1H, $J_{2,3}$ 5.5 Hz, $J_{3,4}$ 2.8 Hz, H-3), 3.94 (broad t, 1H, H-2′), 3.84 (d, 1H, $J_{3',4'}$ 3.4 Hz, H-4′), 3.80 (s, 1H, H-4), 3.80-3.64 (m, 5H, H-2, H-5a, H-5e, H-6′a, H-6′b), 3.58 (dd, 1H, $J_{2',3'}$ 10.3 Hz, H-3′), 3.48 (t,

1H, $J_{5',6'a} \approx J_{5',6'b}$ 6.5 Hz, H-5′), 1.96 (dd, 3H, $^2J_{^1H,^{13}C}$ 6.2 Hz, $^1J_{^1H,^{13}C}$ 128.8 Hz, $NH^{13}CO^{13}C\underline{H}_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 174.19 16 (d, $^1J_{^{13}C,^{13}C}$ 54.49 Hz, $NH^{13}\underline{C}O^{13}CH_3$), 139.84, 139.75, 139.15 (Ph quaternary), 129.42, 129.36, 129.26, 128.94, 128.83 (Ph methine), 103.34 (C-1′), 100.86 (C-1), 78.14 (C-3), 77.70 (C-2), 76.70 (C-3′, C-5′), 74.55 ($Ph\underline{C}H_2$), 73.28 (C-4, Ph$\underline{C}$H2), 71.39 ($Ph\underline{C}H_2$), 69.57 (C-4′), 63.79 (C-5), 62.45 (C-6′), 54.87 (C-2′), 23.18 (d, $NH^{13}CO^{13}\underline{C}H_3$).

ESMS m/z = 648 $[M+Na]^+$ and 626 $[M+H]^+$ ($C_{32}{}^{13}C_2H_{41}O_{10}N$ requires m/z = 625).

3-O-(2-(1,2 -^{13}C)acetamido-2-deoxy-β-D-galactopyranosyl)-D-lyxopyranose (**47**). The procedure followed was similar to that reported for compound **37**. ^1H NMR (500 MHz, D_2O): δ_H 4.92 (d, 1H, $J_{1,2}$ 5.0 Hz, H-1, α), 4.84 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1, β), 4.63 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1′), 4.62 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1′), 2.04 (dd, 3H, $^2J_{^1H,^{13}C}$ 6.2 Hz, $^1J_{^1H,^{13}C}$ 127.8 Hz, $NH^{13}CO^{13}C\underline{H}_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 176.00, 175.90 (d, $^1J_{^{13}C,^{13}C}$ 49.21 Hz, $NH^{13}\underline{C}O^{13}CH_3$), 103.75 (C-1′), 94.93 (C-1, α), 93.09 (C-1, β), 23.15 (d, $NH^{13}CO^{13}\underline{C}H_3$).

ESMS m/z = 378 $[M+Na]^+$ and 356 $[M+H]^+$ ($C_{11}{}^{13}C_2H_{23}O_{10}N$ requires m/z = 355).

4-O-(2-(1,2-^{13}C)acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-1,2,3,6-tri-O-acetyl-β-D-(1-^{13}C)galactopyranose (**50**). The procedure followed was similar to the one reported for compound **40** $[\alpha]_D$ +7.8° (*c* 1, $CHCl_3$), ^{1}HNMR (600 MHz, $CDCl_3$): δ_H 5.84 (dd, 1H, $J_{2',3'}$ 11.3 Hz, $J_{3',4'}$ 3.4 Hz, H-3′), 5.66 (dd, 1H, $J_{1,2}$ 8.3 Hz, $^1J_{^1H,^{13}C}$ 166.7 H z, H-1), 5.65 (dd, 1H, $^2J_{NH,^{13}C}$ 3.3 Hz, $J_{NH,2'}$ 7.1 Hz, $N\underline{H}^{13}CO^{13}CH_3$), 5.37 (ddd, 1H, $^2J_{H,^{13}C}$ 6.6 Hz, $J_{2,3}$ 10.7 Hz, H-2), 5.36 (d, 1H, H-4′), 5.09 (d, 1H, $J_{1',2'}$ 8.1 Hz, H-1′), 4.98 (ddd, 1H, $^3J_{^1H,^{13}C}$ 1.1 Hz, $J_{3,4}$ 2.8 Hz, H-3), 4.30 (dd, 1H, $J_{5,6a}$ 4.7 Hz, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.20 (dd, 1H, $J_{5,6b}$ 6.8 Hz, H-6b), 4.16 (d, 1H, H-

4), 4.04 (d, 2H, $J_{5',6'a} \approx J_{5',6'b}$ 6.7 Hz, H-6'a, H-6'b), 3.89 (t, 1H, H-5'), 3.87 (dt, 1H, $^{3}J_{H,^{13}C}$ 1.5 Hz, H-5), 3.40 (m, 1H, H-2'), 2.12, 2.09, 2.08, 2.04, 2.02, 2.01, 1.97 (s, 21H, OCOC$\underline{H}_3$), 1.97 (dd, 3H, $^{2}J_{1_{H,^{13}C}}$ 6.1 Hz, $^{1}J_{1_{H,^{13}C}}$ 128.3 Hz, NH^{13}CO^{13}C$\underline{H}_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 171.30, 171.19 (C=O), 171.16 (d, $^{1}J_{^{13}C,^{13}C}$ 51.2 Hz, NH$^{13}\underline{C}$O^{13}CH$_3$), 170.62, 170.49, 170.14, 169.81, 169.50 (C=O), 99.13 (C-1'), 92.04 (^{13}C-1), 73.51 (C-4), 73.30 (C-3), 73.09 (C-5), 72.85 (C-2), 70.57 (C-5'), 68.17 (C-3'), 66.97 (C-4'), 63.02 (C-6), 61.51 (C-6'), 53.55 (C-2'), 23.46 (d, NH^{13}CO$^{13}\underline{C}$H$_3$), 21.18 to 20.16 (7, OCO$\underline{C}$H$_3$).

ESMS (high res) m/z = 703.218690 $[M+Na]^+$ (Calcd 703.216548 for $C_{25}{}^{13}C_3H_{39}NO_{18}Na$).

4-O-(2-(1,2-^{13}C)acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-α-D-(1-^{13}C)galactopyranosyl trichloroacetimidate (**53**). The procedure followed was similar to that reported for compound **43**. The first step was the removal of the anomeric acetate to give crude **52** followed by the second step for formation of imidate **53**. ^{1}H NMR (600 MHz, $CDCl_3$): δ_H 8.61 (s, 1H, C=N$\underline{H}$), 6.48 (dd, 1H, $J_{1,2}$ 3.6 Hz, $^{1}J_{1_{H,^{13}C}}$ 179.9 Hz, H-1), 5.81 (dd, 1H, $J_{2',3'}$ 11.3 Hz, $J_{3',4'}$ 3.4 Hz, H-3'), 5.67 (d, 1H, $^{2}J_{NH,^{13}C}$ 3.3 Hz, $J_{NH,2'}$ 7.2 Hz, N$\underline{H}$^{13}CO^{13}CH$_3$), 5.53 (dd, 1H, $J_{2,3}$ 10.87 Hz, H-2), 5.36 (d, 1H, H-4'), 5.33 (dd, 1H, $J_{3,4}$ 2.4 Hz, H-3), 5.07 (d, 1H, $J_{1',2'}$ 8.2 Hz, H-1'), 4.33-4.27 (m, 3H, H-4, H-5, H-6a), 4.16 (dd, 1H, $J_{5,6b}$ 8.2 Hz, $J_{6b,6a}$ 12.6 Hz, H-6b), 4.04 (d, 2H, $J_{5',6'a} \approx J_{5',6'b}$ 6.6 Hz, H-6'a, H-6'b), 3.90 (broad t, 1H, H-5'), 3.40 (m, 1H, H-2'), 2.14, 2.12, 2.03, 2.01, 2.00, 1.98 (s, 21H, COC$\underline{H}_3$), 1.94 (dd, 3H, $^{2}J_{1_{H,^{13}C}}$ 6.1 H z, $^{1}J_{1_{H,^{13}C}}$ 128.2 Hz, NH^{13}CO^{13}C$\underline{H}_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 170.63 (d, $^{1}J_{^{13}C,^{13}C}$ 49.73 Hz, NH$^{13}\underline{C}$O^{13}CH$_3$), 99.24 (C-1'), 92.25 (^{13}C-1), 23.55 (d, NH^{13}CO$^{13}\underline{C}$H$_3$).

ESMS (high res) m/z = 804.118880 $[M+Na]^+$ (Calcd 804.115616 for $C_{25}{}^{13}C_3H_{37}N_2O_{17}Na\ Cl_3$).

Allyl 4-O-(2-(1,2-^{13}C)acetamido-(1,2-^{13}C)-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-(1-^{13}C)galactopyranoside (**54**). The procedure followed was similar to the one reported for compound **44.** $[\alpha]_D$ -14.3° (*c* 0.8, $CHCl_3$), ^{1}H NMR (600, MHz, $CDCl_3$): δ_H 5.92 (dd, 1H, $J_{2',3'}$ 11.3 Hz, $J_{3',4'}$ 3.4 Hz, H-3′), 5.84 (dddd, 1H, $J_{c,d}$ 4.9 Hz, $J_{c,e}$ 6.1 Hz, $J_{b,c}$ 10.4 Hz, $J_{a,c}$ 17.2 Hz, H-c), 5.69 (dd, 1H, $^2J_{NH,^{13}C}$ 3.3 Hz, $J_{NH,2'}$ 6.9 Hz, N<u>H</u>COCH$_3$), 5.37 (d, 1H, H-4′), 5.28 (ddd, 1H, $^2J_{1_H,^{13}C}$ 6.4 Hz, $J_{2,3}$ 10.4Hz, H-2), 5.24 (ddd, 1H, $J_{a,d} \approx J_{a,e}$ 1.8 Hz, $J_{a,b}$ 3.4 Hz, H-a), 5.18 (ddd, 1H, $J_{b,d} \approx J_{b,e}$ 1.6 Hz, H-b), 5.12 (d, 1H, $J_{1',2'}$ 8.2 Hz, H-1′), 4.92 (ddd, 1H, $^3J_{1_H,^{13}C}$ 1.1 Hz, $J_{3,4}$ 2.9 Hz, H-3), 4.48 (dd, 1H, $^1J_{1_H,^{13}C}$ 160.6 Hz, H-1), 4.36 (m, 1H, H-d), 4.32-4.24 (m, 2H, H-6a, H-6b), 4.11 (d, 1H, H-4), 4.09 (m, 1H, H-e), 4.03 (d, 2H, $J_{5',6'a} \approx J_{5',6'b}$ 6.6 Hz, H-6′a, H-6′b), 3.89 (t, 1H, H-5′), 3.71 (dt, 1H, $^3J_{H,^{13}C}$ 1.6 Hz, $J_{5,6a} \approx J_{5,6b}$ 6.0 Hz, H-5), 3.32 (m, 1H, H-2′), 2.12, 2.09, 2.05, 2.02, 2.01, 1.97 (s, 21H, OCOC<u>H</u>$_3$), 1.97 (dd, 3H, $^2J_{1_H,^{13}C}$ 6.0 Hz, $^1J_{1_H,^{13}C}$ 128.2 Hz, NH^{13}CO^{13}C<u>H</u>$_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 171.34 (C=O), 171.33 (d, $^1J_{^{13}C,^{13}C}$ 50.7 Hz, NH^{13}<u>C</u>O^{13}CH$_3$), 170.56, 170.48, 170.16, 169.75, 169.56 (C=O), 133.54 (OCH$_2$<u>C</u>H=CH$_2$), 117.54 (OCH$_2$CH=<u>C</u>H$_2$), 99.91 (^{13}C-1), 98.93 (C-1′), 73.27 (C-4), 73.00 (C-3), 72.20 (C-5), 70.48 (C-5′), 70.07 (O<u>C</u>H$_2$CH=CH$_2$), 69.52 (C-2), 68.06 (C-3′), 67.05 (C-4′), 63.20 (C-6), 61.52 (C-6′), 53.74 (C-2′), 23.51 (d, NH^{13}CO^{13}<u>C</u>H$_3$), 20.65 to 20.20 (6 OCO<u>C</u>H$_3$).

ESMS (high res) m/z = 701.237740 $[M+Na]^+$ (Calcd 701.237284 for $C_{26}{}^{13}C_3H_{41}NO_{17}Na$).

Allyl 4-O-(2-(1,2-^{13}C)acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-(1-^{13}C)galactopyranoside (**55**). The procedure followed was similar to the one reported for compound **45.** $[\alpha]_D$ -10.6° (*c* 1, CH_3OH), ^{1}H NMR (600 MHz, CD_3OD): δ_H 5.94

(ddd, 1H, $J_{c,d} \approx J_{c,e}$ 5.6 Hz, $J_{b,c}$ 10.6 Hz, $J_{a,c}$ 16.9 Hz, H-c), 5.31 (ddd, 1H, $J_{a,d} \approx J_{a,e}$ 1.6 Hz, $J_{a,b}$ 3.3 Hz, H-a), 5.14 (ddd, 1H, $J_{b,d} \approx J_{b,e}$ 1.5 Hz, H-b), 4.64 (d, 1H, $J_{1',2'}$ 8.6 Hz, H-1′), 4.31 (dddd, $J_{d,e}$ 13.0 Hz, H-d), 4.24 (dd, 1H, $^1J_{1_H,13_C}$ 160.3 Hz, $J_{1,2}$ 7.9 Hz, H-1), 4.13 (dddd, 1H, H-e), 4.02 (d, 1H, $J_{3,4}$ 3.1 Hz, H-4), 3.87 (dd, 1H, $J_{5',6'a}$ 7.3 Hz, $J_{6'a,6'b}$ 11.3 Hz, H-6′a), 3.86 (m, 1H, H-2′), 3.80 (dd, 1H, $J_{5,6a}$ 8.1 Hz, $J_{6a,6b}$ 11.3 Hz, H-6a), 3.76 (d, 1H, $J_{3',4'}$ 3.3 Hz, H-4′), 3.70 (dd, 1H, $J_{5,6b}$ 4.2 Hz, H-6b), 3.62 (dd, 1H, $J_{5',6'b}$ 5.5 Hz, H-6′b), 3.61 (m, 1H, H-3), 3.59 (dd, 1H, $J_{2',3'}$ 10.4 Hz, H-3′), 3.52 (m, 1H, H-5), 3.50 (m, 1H, H-5′), 3.46 (m, 1H, H-2), 2.02 (dd, 3H, $^2J_{1_H,13_C}$ 6.0 Hz, $^1J_{1_H,13_C}$ 128.2 Hz, $NH^{13}CO^{13}C\underline{H}_3$).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 171.34 (C=O), 171.33 (d, $^1J_{13_C,13_C}$ 50.7 Hz, $NH^{13}\underline{C}O^{13}CH_3$) 135.55 ($OCH_2\underline{C}H=CH_2$), 117.52 ($OCH_2CH=\underline{C}H_2$), 104.85 (C-1′), 103.91 (^{13}C-1), 78.26 (C-4), 76.90 (C-5), 75.57 (C-5′), 74.89 (C-3), 74.65 (C-3′), 72.37 (C-2), 71.06 ($O\underline{C}H_2CH=CH_2$), 69.57 (C-4′), 62.65 (C-6), 61.37 (C-6′), 55.51 (C-2′), 23.51 (dd, $NH^{13}CO^{13}\underline{C}H_3$).

ESMS (high res) m/z = 427.192730 $[M+H]^+$ (Calcd 427.191951 for $C_{14}{}^{13}C_3H_{30}NO_{11}$).

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